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THE JEKYLL–HYDES OF MYCOLOGY [1]

CHESTER W. EMMONS

Sectorial mutation, loss of ability to produce spores and the multiplicity of species, attest to the lability of the fungi. The size and morphological complexity of fungi reveal at once to the observer many overt aspects of variability and the physiologist easily detects other mutants which are masked by a common phenotype. The consequences and potentialities of the lability of fungi in didactic mycology, in use of fungi as research tools, in plant pathology, and in industrial mycology, are too many and varied to enumerate before an audience already familiar with them. I shall discuss those aspects of the adaptability of fungi which enable them, under appropriate conditions, to turn immediately from a saprophytic existence to parasitism of animal tissues. I submit that this aspect of the adaptability of the fungi places upon mycologists a responsibility which has been only partially acknowledged. Medical mycology should be the concern of mycologists generally and not a subspecialty of medical bacteriology or medicine.

Specialization and complexity among the fungi are spectacularly manifested by those molds and yeasts which flourish under generally inhospitable environmental conditions, grow on substrates unavailable to most microorganisms or play sometimes beneficial, sometimes lethal roles in the economy of nature. Many of the fungi which are found in unusual habitats are now fixed in their present relationships. Some *require* special substrates, some are obligate parasites which require for survival

[1] Presidential address, presented at the Stillwater, Oklahoma, meeting of the Mycological Society of America, August 30, 1960.

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two unrelated alternate hosts, while others are such highly specialized symbionts that they are unable to survive independent existence. These fungi lost their adaptability in an obscure evolutionary past characterized, no doubt, by the disappearance of less successful variants and mutants which failed to establish specialized ecological niches.

Other fungi remain versatile and are able to live under humid conditions yet survive dehydration, to grow at temperatures varying in some cases from 15 to 50° C, to utilize many carbon and nitrogen sources and to grow and reproduce indefinitely as saprophytes and yet they retain the ability to produce lethal disease in man. The latter fungi include molds and yeasts which compete successfully with the rich and varied microflora and fauna of soil and organic debris, are true saprophytes and yet are potential parasites. Perhaps similar versatility exists among plant pathogens but the ecological relationships of fungal pathogens of man are quite unlike those of plant pathogens whose spores regularly overwinter in soil and, as regularly, attack the new growth of plants the following spring. The potential pathogens of man are indefinitely at home in soil and organic debris. When the conidia which they produce freely in these saprophytic environments are inhaled by man, or are introduced by thorns to subcutaneous tissue, the erstwhile saprophyte becomes a parasite possessing temperature tolerance, morphologic lability, and all other characteristics necessary for invasion of the tissues and organs of man and animals.

The diseases these fungi cause may be mild, but after heavy exposure to inhaled spores or in abnormally susceptible persons they may be fatal. These systemic mycoses are not contagious. The fungi which cause them are not dependent for survival upon either immediate, delayed or periodic transmission to another host. The mammalian host, in fact, probably constitutes a "dead-end street" for most of the progeny of the original spores inhaled. Even deliberate attempts to recover these pathogens of man and animals from the buried carcasses of experimentally infected animals have failed. Let me repeat for emphasis that these fungi live indefinitely under natural conditions as true saprophytes. If you will permit me to use the term with neither teleological nor disparaging connotations, I should like to refer to these fungi as *versatile opportunists*. If the term is not entirely appropriate, it emphasizes, nevertheless, a concept which is essential to an understanding of the epidemiology of the deep mycoses.

If you will make a further concession and permit a biographical note, I will illustrate one historical aspect of Medical Mycology which is pertinent to this consideration of the saprophytic occurrence of fungal

pathogens of man. Mycologists' neglect of these fungi has adversely affected the development of medical mycology. Fortunately, this neglect has been corrected in part during recent years. Thirty-one years ago I began a post-doctorate career in the study of fungi of medical importance. By good fortune and by choice, my college and university training had been under three eminent and excellent botanists. Prof. Fred W. Emerson taught morphology, dynamic ecology and their relationships to taxonomic botany in a manner which inspired many of his students to continue investigations at the graduate level. He did not teach us mycology but he aroused in me an intense interest in it. Prof. George W. Martin, during a too brief year, taught me mycology with the inspiration and enthusiasm which his students remember gratefully with a desire to emulate. His courses were stimulating and excellently organized but he taught us no medical mycology. My predoctorate training was continued under Prof. Robert A. Harper, who, during his last year before retirement, still bore the aura of the great early days when epochal discoveries of fungus life histories, the significance of specialized functional organs, the early ground-work of fungus genetics and the economic importance of fungi rewarded the competent and alert mycologist. His course in the application of meticulous cytological methods could have led any student into a fascinating career in morphological and cytological studies of the fungi, but he taught us no medical mycology. I remember well, and still with slight shock, his rather incredulous remark, "Why do you want to study those abortive and uninteresting medical fungi?" Neverthelesss, in 1929, he recommended me for a position, offered by Dr. J. G. Hopkins at the Columbia-Presbyterian Medical Center, where Rhoda Benham had gone from Barnard a few years before to establish a strong diagnostic and research laboratory for Medical Mycology.

In this excursion into autobiography, it is not my intention to criticize three eminent and stimulating teachers and I hope it does not appear that I have been so presumptuous as to do so. On the contrary, I cite my experience in three departments as being representative of the general neglect of an important aspect of mycology. The orphaned science of Medical Mycology was not formally taught in any of the mycology departments of United States universities, so far as I know, before Prof. Arthur T. Henrici began teaching the subject in connection with his bacteriology courses in the late 1920's.

It is a deplorable fact that Medical Mycology developed in the United States for nearly 50 years with little oversight from professional mycologists. Some of the reasons for this neglect are apparent. The liaison between botanist and physician, so close in the early days of medicine,

no longer existed. Many physicians expected from the mycology laboratory the same type of identification service they obtained from the bacteriological technician in the diagnostic hospital laboratory. This was not adequate in an undeveloped field which needed collaboration in research on an equal basis between mycologist and physician. Furthermore, the mycologist was accustomed to work with fungi which presented no known hazard and neither his techniques nor the safety facilities of his laboratory permitted safe handling of some of the virulent pathogens of man. Medical Mycology, except in a very few medical centers of the world, was supported not at all or only at the technician's level. The hospital technician (or the physician) supplemented his bacterial training with self-education in mycology, by study in a hospital laboratory abroad or (in recent years) by attendance at short and intensive training courses in mycological techniques where he gained acquaintance with a few selected fungi. Fortunately, since the establishment in 1926 at Columbia University of the first full-time diagnostic and research laboratory for Medical Mycology in the United States, several excellent training centers now exist.

Historically, the dermatologists had fostered Medical Mycology because of the importance of the almost ubiquitous dermatophytoses or ringworm diseases of the skin. Sabouraud's (**28**) monumental studies and publications in this field still guide and challenge the medical mycologist. Many of the systemic mycoses have important skin manifestations and these "deep mycoses" also attracted the dermatologist who shared this interest with the internist and pathologist. During two decades, at the turn of the century, physicians, especially internists, epidemiologists and pathologists, in an imposing series of papers, reported several new and important systemic mycoses. Some of these papers are still remarkable for their insight and accurate reporting. It is true that *Histoplasma* and *Coccidioides* were described, named and reported as protozoa. Perhaps a collaborative study between mycologist and physician, making available to the former both the parasitic growth form of the fungus in tissue and its saprophytic form in culture, would have led to a more accurate identification.

The noteworthy papers published between 1890 and 1910 were by no means the earliest classics in the literature of Medical Mycology. Rénon's 1897 monograph (**26**) on aspergillosis is surprisingly comprehensive and little has been added to our knowledge of pulmonary aspergillosis during the 63 years since its publication. Forty-four years earlier Robin (**27**) had written a text which should be read by every mycologist interested in the history of medical mycology. The antiquity

of this specialty merits a brief review of still earlier studies but I can do no more than mention the oft cited investigations of oral thrush and of various types of ringworm reported by Langenbeck, Mueller, Gruby, Remak, Lebert, Malmsten and Robin between 1839 and 1848.

For the earliest recognition of a parasitic relationship of fungi to animal life, we must turn to entomology. As early as 1794 Torrubia, a Franciscan friar, fancifully described and illustrated *Cordyceps,* recognizing its attachment to insect larvae. His imaginative drawings shows a tree-like *Cordyceps* growing from a subterranean larva, while an adult insect flies overhead bearing a smaller specimen of the same fungus. Gray (**19**) described this fungus more accurately 100 years later. DeGeer in 1782 described the common mycosis of the housefly and Cohn (7) in 1855 named the etiologic agent *Empusa muscae.* Muscardine was perhaps the first generally acknowledged disease of silkworms. Bassi de Lodi (**4**) demonstrated in 1835 that this disease was caused by a fungus, Balsamo in 1836 named the fungus *Botrytis bassiana* and Audouin (**2**), in the following year, reported experimental production of the disease by inoculation with the fungus.

Having traced Medical Mycology back to its recognizable origins, let us now return to the present and attempt to evaluate the importance of this branch of mycology. Vital Statistics of the United States lists yearly between 350 and 450 deaths from mycoses. In some of these, even though a fungus was the terminal cause of death, the patient probably was debilitated following an earlier primary disease. On the other hand it is generally assumed, and on good grounds, that many additional persons die each year from undiagnosed mycoses under circumstances in which clinical acumen and laboratory facilities were lacking. Whether mycotic deaths in the reporting area of the United States total 300 or 1000, this number is small when compared with deaths from some of the major causes. However, reported deaths from mycoses during 1957, the latest year for which data are available, totaled 369 (**35**). This exactly equaled the number of poliomyelitis deaths reported in the United States for the same year and exceeded by one the total number of deaths reported for whooping cough, diphtheria, scarlet fever, typhoid, malaria and brucellosis. Although deaths from bacterial diseases have been reduced by antibiotic therapy to a fraction of their former totals, the mycoses remain difficult to treat. Amphotericin, an antibiotic produced by Squibb, has been the drug of choice in treatment of several mycoses, but it is only partially successful (**17, 32**). I have had under experimental test for four years a new antibiotic produced by Hoffman-La Roche, Inc. and we have now had this drug on clinical trial for a year

(**15, 20, 34**). It shows promise of therapeutic value in several mycoses, but we continue to look for more effective drugs. The poor response of mycoses to therapy represents one aspect of their medical importance.

The mortality data on mycoses may be inaccurate, but a much greater discrepancy exists between numbers of infections and fatalities. Within certain endemic areas of our arid Southwest, few residents escape coccidioidomycosis (**8, 31**). One patient was infected enroute through this area when she stepped off the train for a few moments in a city within the endemic area. In many areas of Eastern and Central United States 50–90 per cent of residents react to histoplasmin, indicating that they have at some time had this systemic mycosis (**6, 16, 24, 25**). Fortunately, most of these cases of potentially fatal mycoses are mild and self limited. Indeed, many of them are entirely asymptomatic and evidence that an infection has occurred may rest solely upon hypersensitivity to an antigen injected intradermally. For most of the other mycoses, we are unable to gather any epidemiological data concerning the occurrence of mild forms. Knowing that some of the potential pathogens are commonly in man's environment, one can assume that their spores are frequently inhaled.

Experimental infection of animals, epidemiological data from numerous outbreaks of mycoses in groups exposed to spores, and many clinical studies indicate that at least three factors influence the severity of mycoses in man. In the normal individual inhalation of a few conidia may produce only an inapparent infection while inhalation of a great many spores may be followed by a mild, severe or fatal mycosis. A second factor is that of racial susceptibility. Negroes, Mexicans, Filipinos and other dark-skinned races are more susceptible to coccidioidal granuloma than whites. On the other hand innate immunity to this mycosis is most obvious in white females in whom the classic entity of "Valley fever" is most often seen (**31**). A third factor is abnormal susceptibility. Progressive, fatal cryptococcosis is often associated with Hodgkin's Disease or other malignant lymphatic condition. On the other hand, healed pulmonary lesions of cryptococcosis have been found in persons who presumably have a strong immunity against *Cryptococcus* (**23**). Phycomycosis is most often seen in diabetics (**3, 21, 22**). These three factors which are known to influence the severity of mycoses only partially explain the relative impunity with which man lives among pathogenic fungi.

There are two dozen well-defined mycoses of man in which more than 60 fungi play the etiologic roles. If one counts the unusual infections, the clinical rarities and the fungi which cause infection exceptionally,

these numbers can be doubled. Medical literature records many additional cases which need confirmation before acceptance as actual mycotic infections. Without systematically discussing the mycoses, I shall remind you only that *Coccidioides immitis, Nocardia asteroides, Histoplasma capsulatum, Cryptococcus neoformans, Aspergillus fumigatus, Absidia corymbifera* and species of *Rhizopus* can cause fatal mycoses and that they can be isolated easily and regularly from saprophytic habitats. Other fungi which cause potentially fatal disease are assumed on good evidence to have a saprophytic existence and several fungi which cause chronic but rarely fatal disease have been isolated from man's environment (**1**, **10**, **11**, **12**, **13**, **14**, **16**, **18**, **30**, **33**, **36**).

I shall discuss only three of the more familiar saprophytes which sometimes play a lethal role. If I appear to be an alarmist I assure you it is intentional. My purpose is to emphasize the fact that Medical Mycology does not deal with a bizarre group of fungi but with molds and yeasts with which we may be in almost daily contact; that Medical Mycology is not an esoteric specialty but is "part and parcel" of mycology; that mycologists must continue to take increasing responsibility in the study of the fungi which have in the past been left to a few specialists, to the bacteriologists, to the physician and to the laboratory technician.

Pulmonary aspergillosis has been observed, but rarely, in many species of mammals. It is an important cause of death in chicks and other young birds. It is one of the causes of brooder pneumonia and the fungus grows so profusely that it lines the air sacs of the bird with a green felt and penetrates associated tissues. Aspergillosis is an important cause of death in many water birds and the emperor penguin, when brought to zoos in the United States, almost invariably succumbs to it within weeks or months despite precautions to guard the birds from exposure to *A. fumigatus* conidia. It is apparent that man and other mammals are usually resistant to progressive, fatal, pulmonary aspergillosis, unless conditioned by a primary disease, that the adults of many but not all species of birds are relatively resistant and that young birds of several species are susceptible to severe epidemics of this mycosis.

Aspergillosis is not solely a disease of the lung. *A. fumigatus* may be allergenic without invasion of tissues, it may be traumatically implanted in the eye and cause a corneal ulcer, it may invade the sinuses, it (with *A. flavus*) causes a destructive disease of the honey bee and it is one of the important causes of mycotic abortion in cattle and sheep. The fungus is abundant in certain habitats, it grows rapidly and sporulates profusely, it produces one or more endotoxins and it has demonstrated ability to invade tissues widely and destructively. It is surpris-

ing perhaps that it is not found more often as a pulmonary pathogen of man than the number of reported cases indicate.

Rénon in 1893 prepared a doctoral dissertation on aspergillosis and in 1897 expanded this into a monograph in which he reported his own observations of this disease, and reviewed to 1897 the report of others (**26**). Four of the five cases which he observed and recognized as primary aspergillosis were in persons who professionally fed pigeons by masticating grain and forcing it directly from the mouth into the beak of the pigeon. The fifth was in a patient engaged in the task of cleaning hair for wigs with flour. Rénon believed the sources of infection in these cases were the grain and flour and he verified the presence of *Aspergillus fumigatus* in these materials. Since he isolated *Mycobacterium tuberculosis* rarely and with difficulty from other cases of aspergillosis, we may question (as did some of his contemporaries) whether there was another primary disease in the five cases he classified as primary aspergillosis. Despite the excellence of Rénon's early paper on aspergillosis, the two types of 19th century industrial exposure he emphasized are no longer significant in the epidemiology of aspergillosis. An industrial exposure may exist among farmers and gardeners and probably some of the cases of "farmers' lung," an allergic disease which has received particular attention in England and Europe, follow exposure to *A. fumigatus*.

A. fumigatus is thermophilic and it grows abundantly on vegetation under certain conditions of biological "heating." The care and maintenance of trees and shrubs along city streets and country roads commonly involves use of a "chipper" which reduces green branches, twigs and leaves to a coarse type of mulch prized by gardeners. Fermentation of a pile of this green material, especially if it is further moistened by rain, produces a high temperature which, with the degree of aeration present among the coarse particles, may support a profuse and apparently almost pure growth of *A. fumigatus*. Other types of composting vegetation may also support the fungus and provide opportunities for exposure of birds and animals to conidia, but no more profuse and extensive growth of this pathogenic fungus has been reported, so far as I know, from other habitats.

The Phycomycetes may well be taken as a second example of a group of fungi familiar to every mycologist, diverse in form and habitat, growing as saprophytes on dung, vegetation and other organic debris and containing species which are pathogens of plants, as well as of man. Mycoses caused by *Absidia corymbifera* were recognized 75 years ago but the frequency with which the phycomycoses occur and the diversity

of genera and species involved are only now being recognized (**3**, **22**, **23**). Besides *A. corymbifera*, several species of *Mucor* and *Rhizopus* have been incriminated as the etiologic agent of disease. These mycoses are rare but when they do occur, rapid invasion of the central nervous system leads to an early fatal termination. *Mortierella* has been isolated from subcutaneous lesions and *Basidiobolus ranarum* has been proved to be the etiologic agent of subcutaneous infections in Indonesia. Many, but not all, of these infections have been in diabetics and it is evident that diabetes increases the susceptibility of man to infection. Finally, the virulent pathogen *Coccidioides immitis*, in my opinion, is a Phycomycete (**9**).

I shall conclude with a discussion of cryptococcosis. The etiologic agent, *Cryptococcus neoformans*, can not be characterized, as were the preceding examples, as a fungus familiar to mycologists because of its ubiquity, but one constant and abundant source in man's environment has been overlooked until recently. Sanfelice isolated this fungus from peach juice at about the same time in 1894 that it was found in a patient where it caused a lesion of the tibia and overlying tissues (**5**, **29**). Since that time it has been found, usually in fatal disease of the central nervous system, in many instances in man and animals. It is the most frequent cause of mycotic meningitis but a primary pulmonary lesion has been found in many cases of this type of meningitis and it is assumed that infection follows inhalation of the fungus and that the pulmonary lesion is usually transient. The observed presence of solitary or healed pulmonary lesions in a few otherwise healthy persons supports this concept but widely disseminated lesions sometimes occur. *C. neoformans* causes disease in horses, cats, monkeys and other animals, as well as in man. It has been isolated from pooled samples of milk and two large epidemics of cryptococcal bovine mastitis have been reported.

For 60 years after its isolation, *C. neoformans* was known only in association with the tissues, surfaces, secretions or excretions of man and animals. In 1950 I reported its isolation from barnyard soil and in 1955 found it was very frequently present in the old nests and in droppings under roosting sites of pigeons (**12**). This association has been confirmed by other investigators in other parts of the world and it appears to be constant and widespread. The fungus is as readily found on window ledges of office buildings and beneath roosting sites in open attics of old buildings in cities as in barns and haymows on farms. These are virulent strains of the fungus, indistinguishable from those isolated from fatal cases of cryptococcal meningitis. The public health implications of this habitat of a fungus capable of causing a severe menin-

gitis which is resistant to treatment are obvious. *C. neoformans* exemplifies, as do *Aspergillus* and the Phycomycetes, the phenomenon of a virulent fungus, often abundantly present in man's environment as a saprophyte. We can only assume that man's resistance to *Cryptococcus*, unless overcome by a conditioning primary lymphomatous disease, protects him from a fatal infection.

The role of another factor in development of a mycosis after exposure, namely, intensity of exposure, is difficult to evaluate for cryptococcosis at the present time. Lack of a diagnostic skin test has prevented us from learning the extent and frequency of subclinical or mild cryptococcosis. In most of the diagnosed cases, the disease appears in a fatal meningeal form. Most cases of coccidioidomycosis and histoplasmosis are mild and self-limited. No such pattern of disease has been established for cryptococcosis but it is logical to suppose that its epidemiological pattern is similar. There is, in fact, considerable circumstantial evidence that this is the case. There are records of several outbreaks of pneumonitis in men who cleaned or demolished old buildings in which pigeons had roosted and nested (**16**). These men were heavily exposed to dust from pigeon excreta. *C. neoformans* occurs regularly in such material. Although these outbreaks have been diagnosed in retrospect as histoplasmosis, *Histoplasma* has not been isolated from deep deposits of pigeon droppings on the upper floors of buildings. In the few cases where this fungus does appear to be associated with pigeon excreta, the specimens have been collected only at ground level or under conditions in which fecal droppings have been mixed with soil. Therefore, it is very probable that these workmen were heavily exposed to *C. neoformans* in the atmosphere in which they worked. Evidence is circumstantial for the diagnoses of both cryptococcosis and histoplasmosis in epidemics or outbreaks of this type. I have continued to advance the hypothesis that these outbreaks were a previously unrecognized clinical form of cryptococcosis because I think the weight of evidence supports that diagnosis and because if this hypothesis is advanced and sufficiently publicized, it may be possible at some time in the future to adequately consider and test this hypothesis in the diagnosis of a future similar epidemic at the time it occurs.

I hope that my diversions into the epidemiology and clinical variations of some of the mycoses have not obscured but rather have emphasized my central theme. Medical Mycology is an integral part of Mycology and should receive the attention of all teaching mycologists because the etiologic agents of most of the systemic and subcutaneous mycoses are normally saprophytes in man's environment. Exposure by inhalation

or traumatic implantation of conidia must be very frequent indeed and the diversity of clinical manifestations and the numbers of potentially pathogenic fungi are now only partially known.

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KANSAS AEROMYCOLOGY VI: HYPHAL FRAGMENTS[1]

S. M. Pady and C. L. Kramer[2]

(with 2 figures)

INTRODUCTION

The presence of numerous small hyphal pieces in the air is a relatively recent discovery. They have been reported from air over land masses in England (**15**), Canada (**7**) and the Canadian Arctic (**14**). Their presence also over large bodies of water has been observed; over the Arctic Ocean (**16**), the Pacific Ocean (**11**), the Atlantic Ocean (**15**) and recently over the Mediterranean Sea (**20**). In Kansas, hyphal fragments were found to be a constant and characteristic feature, occurring throughout the year with highest numbers during growing seasons (**12**). Many of the hyphae in the air are viable and capable of germination and some have been cultured. This paper presents additional data on their seasonal occurrence and viability.

In 1953, a study of the fungal constituents of the air in Kansas was initiated using a slit sampler. The fungus spore concentration was determined quantitatively throughout the year (**12**) and in 1956 culture techniques were employed with nutrient plates being exposed daily. Data on materials and methods have been published (**8**, **19**). For the quantitative study of hyphal fragments, microscope slides coated with silicone (DC-4) were exposed daily, for 30 minutes, between 8:30 and 9:30 AM on a sampling platform 150 feet above ground. Counts were made under high power and numbers determined on a cubic foot basis. For viability studies, water agar slides were exposed for two minutes in the slit sampler, then incubated for 12–24 hours in a saturated atmos-

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phere at room temperature. The short exposure was necessary because longer exposures caused desiccation of the agar film.

RESULTS AND DISCUSSION

Hyphal fragments were present 364 of the 403 days and were found throughout the year (FIG. 1). Despite their year-round occurrence, they were much more numerous during growing seasons, increasing as growth begins in the spring and decreasing in the fall. During winter, numbers ranged from one to six per cubic foot with a much higher

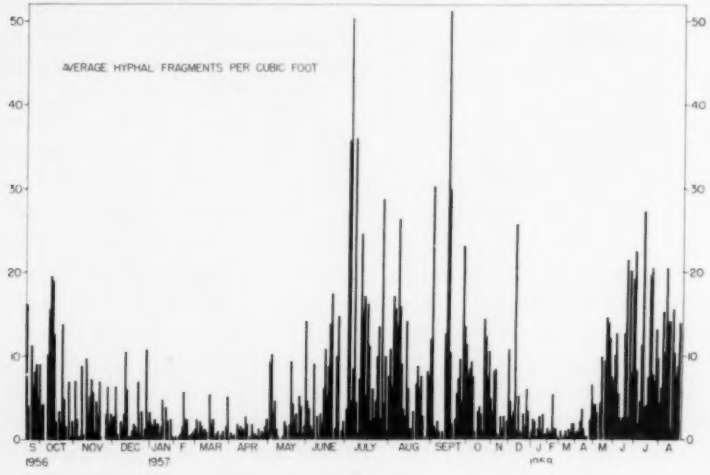

FIG. 1. Numbers of hyphae/cu ft in Kansas air. Daily sample consisted of a single silicone-coated slide exposed in a slit sampler for 30 minutes from the roof of Willard Hall, Kansas State University campus.

number occasionally. On February 25, 1958, the concentration of fungus spores was one of the lowest ever recorded, 0.7/cu ft, with only 5 spores on the entire slide, yet there was a single hypha on the slide. During the summer, hyphal numbers were usually from 5 to 20/cu ft and on September 16, 1957, a peak concentration of 51.3/cu ft was reached. As was true for fungus spores (**8**) there were large day-to-day variations (FIG. 1). September 16–19, 1957, for example, hyphae were 51.3, 30.0, 10.4 and 1.8/cu ft and December 7–13, excluding December 9 when no sample was taken, numbers were 2.1, 1.5, 3.0, 10.5, 6.0 and a trace/cu ft.

Sampling was started in September, 1956, in a drouth period that extended to March, 1957. Then moisture conditions were favorable,

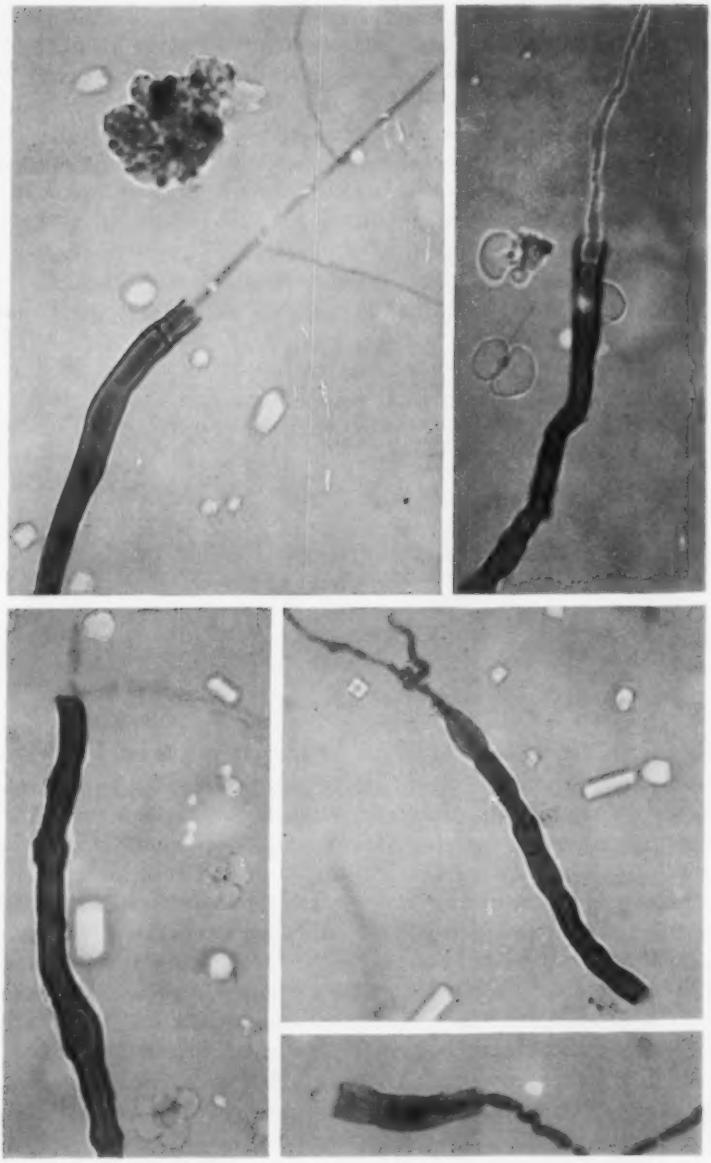

FIG. 2. Photomicrographs of germinating hyphal fragments from the air. Water agar slides were exposed for 2 minutes in a slit sampler and incubated 24 hours.

except during mid-July to mid-August, 1957. This is reflected in FIG. 1, when numbers reached a peak in July, declining in August, and rising again to a second peak in September. Numbers exceeded 20/cu ft five days in July and four in September. In comparison with fungus spores, they rank eighth in numbers, being exceeded only by *Cladosporium*, *Alternaria*, basidiospores, yeasts, smuts, two-celled hyaline, and *Fusarium* spores (**8**).

The hyphal fragments may be long or short, simple or branched, colored or hyaline. The majority of them range from 5 to 15 μ; however, long strands up to 100 μ are occasionally found. Dematiaceous are more numerous than hyaline hyphae. Some typically dark, thick-

TABLE I

NUMBERS OF VIABLE HYPHAE OBTAINED ON WATER AGAR SLIDES EXPOSED IN A SLIT SAMPLER FROM THE ROOF OF WILLARD HALL, K. S. U. CAMPUS: 2-MINUTE EXPOSURE, INCUBATION 12–24 HOURS. 1957

Month	Number days sampled	Days hyphae present	Numbers on slide			Per cent germination
			Min.	Max.	Av.	
June	20	9	1	12	6	54
July	19	17	1	20	6	30
August	23	19	1	16	7	29
September	19	14	1	16	6	57
October	19	15	2	17	6	62
November	12	6	1	3	2	82

walled types are shown in FIG. 2. The fragment frequently consisted of the terminal portion of a conidiophore, and often with a young spore attached. Occasionally a cluster of radiating conidiophores was found. Most fragments were septate but in a few cases nonseptate strands were found.

Because of the short exposure, hyphae were found only on 80 of the 112 water agar slides, and in low numbers (TABLE I) although germination ranged from 29 to 82 per cent. These percentages may not be significant because of the small numbers involved. Typical germination of hyphal sections are shown in the photomicrographs in FIG. 2. Germ tubes were usually formed at the broken ends; in a few rare cases a lateral germ tube developed. The germ tubes were generally narrower than the hyphae, branching often and appeared to be identical to those formed from spores. Germinating fragments may be quite small, consisting of very short pieces with possibly only a single cell (FIG. 2).

An attempt was made to determine identities of the viable hyphal frag-

ments. Isolated germinating fragments were transferred to test tubes containing potato dextrose agar and the colony examined after 5–7 days. To date, hyphal fragments have yielded colonies of *Cladosporium*, *Alternaria*, and *Penicillium*. This work will be continued and reported in detail later.

Hyphal fragments were found by Meier (**10**) on slides exposed by Lindbergh in his trip over the Arctic in 1933. Newman (**11**) exposed slides from an airplane on a trip from New Zealand to the United Kingdom in September, 1947. Hyphal fragments were found on the slides exposed 50, 210, and 700 miles from New Zealand over the Tasman Sea. In the exposure 210 miles from shore there were 26 hyphae and 273 fungus spores.

In England, fungal fragments were observed on slides exposed during a flight from London to Prestwick (**15**), and Last (**9**) at Rothamsted noted their presence in the air near ground level. No mention of hyphal fragments in England is given in the extensive fungal spore studies of the air by Hirst (**5**), Gregory (**2**), Gregory and Hirst (**3**), Hyde and Williams (**6**), Richards (**18**), and Gregory and Sreeramulu (**4**). Sreeramulu (**20**), however, recorded the presence of hyphal fragments in a series of exposures over the Mediterranean Sea from the deck of a ship enroute from London to India. Of 19 slides exposed, 14 bore fungal hyphae, 4 were noted with a question mark, and only one was clearly negative. The average number of hyphae on the 14 slides was 3.7/m^3, compared with 55.8 fungus spores/m^3. The highest number of hyphae was 10.2/m^3 obtained near Gibraltar while the fungal spore concentration was 106/m^3.

Diurnal periodicity of fungal spores was first reported by Hirst (**5**) and by others since that time [Gregory and Sreeramulu (**4**), Waggoner and Taylor (**21**), Panzer et al. (**17**)]. Diurnal periodicity has been found to occur also in hyphal fragments; in a preliminary report with a continuous spore sampler, their numbers were low at night, and rose to a peak in the afternoon (**13**). In a series of studies on spore numbers in Kansas, Bhatti (**1**) found hour-to-hour variation in numbers of fungal hyphae, from May to September, 1957. In June and September, peaks were in the afternoon with minor peaks at night, whereas in May, July, and August, the reverse seemed to be true. More data are needed to determine which pattern is characteristic.

The discovery that airborne hyphal fragments are viable makes their presence of greater importance than hitherto realized. Many aerobiologists have omitted hyphal data because their presence may have been considered to be of no significance. The fact that some of the hyphae

are viable means that they should be included in aeromycological and epidemiological investigations since they can produce colonies and in the case of pathogens could cause infection. They compare with chlamydospores and microsclerotia in their ability to remain viable. In the writers' opinions this method of asexual reproduction of fungi is important and merits further study.

SUMMARY

1. Hyphal fragments were present in Kansas in the air throughout the year with highest numbers during growing seasons.

2. Numbers in winters varied from 1 to 6, in summers from 5 to 51 per cubic foot.

3. Fragments varied in size and length, mostly from 5 to 15 μ, occasionally to 100 μ. The majority were brown septate thick-walled but hyaline fragments were also present.

4. Viability ranged from 29 to 82%. Preliminary isolations produced colonies of *Cladosporium*, *Alternaria*, and *Penicillium*.

5. The abundance of viable fungal fragments suggests that this may be an important means of asexual reproduction.

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CRITICAL NOTES ON SOME PLANT RUSTS III [1]

M. J. Thirumalachar

The recent book entitled "Illustrated Genera of Rust Fungi," published by Dr. G. B. Cummins (**1**), is to be welcomed by all students of rust fungi. It is one of the best books written for the students and research workers alike, and for many years to come, will remain the chief source of information on the taxonomy of Uredinales. In attempting to present the complicated group of rust genera in a simplified manner, Cummins has taken several arbitrary decisions on taxonomic groupings which naturally call for reconsideration and correction. It is true that there is no general agreement on what should be the criteria for separating genera which are acceptable to every one. Our own concepts on rust taxonomy have been changing from time to time as more data become available on the morphology and life-cycle of the organisms. Even so, there should be no faltering in evaluating the status of genera, when they are based on characters which are at present accepted as valid and made use of in distinguishing one genus from the other. Since the publication of this book, the writer has restudied type materials of many of the genera, whose characters appeared to be wrongly interpreted by Cummins, and some of these findings are presented here.

(1) Emended descriptions of rust genera

The accounts of the generic descriptions of several genera were emended by Thirumalachar in the course of the studies of rust genera. These included among others (a) *Nothoravenelia* Diet., (b) *Endophylloides* Whetzel & Olive, and (c) *Anthomycetella* Syd. The original descriptions were based on incorrect interpretation of the developmental morphology of the sorus and spores. In *Nothoravenelia* for instance, the spores were thought to be 2-celled, and in *Endophylloides* the aecioid telia were described by Whetzel and Olive as caeomoid and nonperidiate. The true nature of teliospore heads with abstrictor cells in *Nothoravenelia* was described by the writer (**5**). The peridiate nature of a columnar type of telium in *Endophylloides* was shown (Thirumalachar, **5**). Similarly the teliospore in *Anthomycetella* was shown to be composed of a

[1] Part II in Sydowia **5**: 23–29, 1951.

single layer of teliospores borne on sporogenous basal cells, unlike Sydow's description of multilayered teliospore head (**3**). In the manual published by Cummins the emended descriptions are accepted but accredited to the original authors and without mention of the source of information.

(2) COLEOPUCCINIELLA HARA, SYNONYMOUS WITH CHRYSOMYXA

In an earlier paper, Thirumalachar and Whitehead (**8**) gave an account of the chief characteristics of the genera *Coleopuccinia* Pat. and *Coleopucciniella* Hara. It was pointed out that *Coleopuccinia* has 2-celled teliospores with pedicels gelatinising as in *Gymnosporangium* and bears no resemblance to *Coleopucciniella* with 1-celled teliospores occurring in chains. It was, therefore, surprising that in the manual of rust genera, Cummins treats *Coleopucciniella* as a synonym of *Coleopuccinia*, which evidently is not correct.

Examination of numerous collections of *Coleopucciniella simplex* (Diet.) Hara has shown only teliospores occurring in yellow waxy sori on the lower leaf surface of *Eriobotrya japonica*. The telia are subepidermal, erumpent, the teliospores being cuboid to ovate, 1-celled, developed in chains of 5–10 spores. The telial crust is compact and waxy and the chains show lateral coalescence. The telia are identical with those of *Chrysomyxa* and there are no characters to differentiate it as a separate genus. The following nomenclatural change is proposed.

Chrysomyxa simplex (Diet.) Thirumalachar, comb. nov.

Syn. *Coleopuccinia simplex* Diet., Ann. Mycol. **7**: 355. 1909.
Coleopucciniella simplex (Diet.) Hara, Parasitic Fungi of Japan (In Japanese), p. 262. 1936.

(3) STEREOSTRATUM MAGN. REPLACES CLEPTOMYCES ARTHUR

The genus *Cleptomyces* was described by Arthur in 1918 to accommodate a rust on *Aegiphila* sp. in South America, with subepidermal pycnia and 2-celled pedicellate teliospores which are thick-walled, coloured and *Puccinia*-like. The genus is identical with *Cumminsiella* but differs only in having 4 or more germ pores in each cell of the spore as against 2 lateral germ pores known in the latter. The laminated wall layer which is known in several rust species, including *Puccinia allicepulae* Mundkur and Thirum., is an interesting feature.

The genus *Stereostratum* was described earlier by Magnus in 1899 for a Japanese rust on bamboo, which produced large *Corticium*-like patches. Uredia and telia have been described, the teliospores being

2-celled, *Puccinia*-like and having 3 to 5 germ pores in each cell. In young spores the laminated nature of the wall layer is evident. The occurrence of 3–5 scattered germ pores in each cell of the spore is the distinguishing character, which is duplicated in the later described genus *Cleptomyces*. Cummins (1) treats *Stereostratum* as a synonym of *Puccinia*, which is not correct. The presence of 3–5 germ pores in each cell of the spore itself separates the genus from *Puccinia*. *Cleptomyces* Arth. needs to be treated as a synonym of *Stereostratum* and the description would be as follows:

STEREOSTRATUM P. Magn., in Ber. deutsch. Bot. Ges. **17**: 181. 1899.
Syn. *Cleptomyces* Arth., Bot. Gaz. **65**: 464. 1918.

Pycnia subepidermal, globoid; aecia unknown; uredia subepidermal; urediospores pedicellate; telia subepidermal, erumpent; teliospores 2-celled, *Puccinia*-like, pedicellate, germ pores 3 to 5 in each cell, wall pigmented, germination unknown, but evidently external.

TYPE species: *Stereostratum corticoides* (Berk.) Magn., on bamboo, Japan.

Stereostratum lagerhamianus (Diet.) Thirumalachar, comb. nov.
Syn. *Cleptomyces lagerhamianus* (Diet.) Arth., on *Aegiphila* sp., Ecuador, South America.

(4) DESMOTELIUM SYD. SYNONYMOUS WITH CHRYSOCELIS LAGERH. ET DIETEL

In the Illustrated Genera of Rust Fungi, Cummins (1) has treated *Desmotelium* as a synonym of *Chaconia* Juel. Even earlier, Thirumalachar and Mundkur (7) had pointed out that *Desmotelium* does not possess differentiating characters of its own and may have to be merged with *Chrysocelis*. This is in keeping with the structure of the pycnia which is subepidermal in both the genera. *Chaconia* is distinguished by the presence of subcuticular pycnia and hence *Desmotelium* is not synonymous with it. The following nomenclatural changes have to be made:

Chrysocelis coaetaneum (Syd.) Thirumalachar, comb. nov.
Syn. *Desmotelium coaetaneum* Syd., Ann. Mycol. **35**: 252. 1937.
Chaconia coaetaneum (Syd.) Cumm., Bull. Torrey Bot. Club **87**: 31–45. 1960, on *Millettia rhodantha*, Sierra Leone, Africa.

(5) ON THE GENUS CATENULOPSORA MUNDKUR

The genus was established by Mundkur for a rust on *Flacourtia sepiaria* in India. Only uredia and telia were described, the uredia

being subepidermal and paraphysate. The chief diagnostic character of the genus was the chains of 1-celled teliospores, firmly united, falling apart, and germinating immediately. Germination took place by the prolongation of spore apex and there was no germ pore. The genus is distinctly separate from *Cerotelium* Arth. which in the first place has characteristic phakopsoroid uredia (there being peripheral basally united paraphyses). As pointed out by Mains (**2**) the telial chains are columnar at the base and pulverulent at the apex, or spores separating and germinating away.

The characters of uredia and telia of *Catenulopsora* are, however, very close to *Kuehneola* Magn. The teliospore chains are very similar in there being catenulate spores which are firmly united and fall apart. The uredia in both are not phakopsoroid. The genus was separated because of the concept gained by descriptions of various authors that there is a distinct germ pore in each cell of the spore, and that the promycelium extrudes out of the germ pore. In contrast, there is no germ pore in *Catenulopsora,* the apices of the teliospores forming a beak before germination and later prolongating into promycelium. This was considered as an important character distinguishing the two genera.

In a recent trip through Canada, fresh materials of *Kuehneola albida* (Kuehn) Magn. [*K. uredinis* (Link) Arth.], the type of the genus with telial stages, were collected. A detailed study of these as well as authentic material from various herbaria was made. The uredia are subepidermal and are not phakopsoroid. The teliospore chains are similar to those described in the literature, firm and falling apart. The teliospores at the apex in all cases were protruded into beaks which prolongated into promycelia. Even the illustration given by Cummins (**1**) shows this character. There is no germ pore for the teliospore and germination is by the prolongation of the apical beak. The same characters are duplicated in *Catenulopsora.* Hence it is proposed to merge *Catenulopsora* with *Kuehneola.* The status of other species of *Kuehneola* described at present can be considered only after observing the teliospore germinations.

Three other species of *Catenulopsora* have been described and the following nomenclatural changes are proposed:

Kuehneola flacourtiae (Mundkur) Thirumalachar, comb. nov.

Syn. *Catenulopsora flacourtiae* Mundk., Ann. Bot. N.S. **7**: 217. 1943.

KUEHNEOLA VITIS (Butl.) Syd., Monogr. Ured. **3**: 323. 1914.

Syn. *Catenulopsora vitis* (Butl.) Mundk., Ann. Bot. N.S. **7**: 218. 1948.

Kuehneola zizyphi (Ramakr. & Subram.) Thirumalachar, comb. nov.
Syn. *Catenulopsora zizyphi* Ramakr. & Subram., Curr. Sci. **15**: 261, 262. 1946.

Kuehneola grewiae (Thirum. & Mundk.) Thirumalachar, comb. nov.
Syn. *Catenulopsora grewiae* Thirum. & Mundk., Myc. pap. C.M.I. Kew. **40**: 15. 1951.

(6) STATUS OF THE GENERA BASED ON SUBCUTICULAR OR SUBEPIDERMAL PYCNIA

According to our present knowledge of rust fungi, certain taxonomic values have been assigned to each of the characters. The subcuticular or subepidermal nature of pycnia has the same taxonomic status as peridiate or nonperidiate sori, internal or external basidium, sessile or pedicellate teliospores. This has been accepted and freely made use of by Cummins (**1**). The genus *Cumminisiella* Arthur for instance is differentiated from *Uropyxis* Schroeter, *Tranzschelia* Arthur and *Leucotelium* Tranz. from *Puccinia*, *Achrotelium* Syd. from *Chrysella* Syd. and many others on the subepidermal or subcuticular nature of pycnia, the rest of the characters being the same. Cummins further confirms this feature in the notes on *Scopella* stating "the genus differs from *Uromyces* mainly because of the subcuticular spermagonia."

It is evident that the taxonomic concept cannot be changed without valid reasons. The genus *Kulkarniella* Gokhale & Patel was shown by Thirumalachar and Kern (**6**) to be synonymous with *Monosporidium* Barclay, a genus which was made valid after the discovery of subcuticular pycnia. It differed from *Endophyllum* because of the subepidermal nature of pycnia in the latter. In the manual, however, Cummins (**1**) emends the description of *Endophyllum* Léveille as "spermagonia subepidermal, subcuticular or wanting" and thus includes *Kulkarniella* as a synonym of *Endophyllum*. Lack of pycnia is not a taxonomic character, but only indicates that the investigators have not seen them. The subepidermal and subcuticular condition of pycnia considered to be so important in previously mentioned genera has been overlooked for *Endophyllum*. Since this cannot be done with our present knowledge of the rust fungi, *Kulkarniella* has to be treated as a synonym of *Monosporidium* Barclay, and *Endophyllum* takes care of species only with subepidermal pycnia.

The second genus that comes for consideration is *Hapalophragmiopsis* Thirum. The subcuticular pycnia for the genus *Hapalophragmium* was first discovered by Thirumalachar (**4**) in *H. mysorense* (on *Derris benthamia*). The genus *Hapalophragmiopsis* was separated to accom-

modate forms with subepidermal pycnia. Cummins (**1**), in attempting to merge *Hapalophragmiopsis* with *Hapalophragmium*, has emended the generic description of the latter as "spermogonia subcuticular or rarely subepidermal, but always with a conical form and flat basal hymenium." Since pycnia are known only in one species of *Hapalophragmium* described by the writer (**4**), the question of rarity of subepidermal condition does not arise. The terms "but always with a conical form and flat basal hymenium" are added by Cummins to indicate that they are not flask shaped as in many forms with subepidermal pycnia and hence mere position of sorus with reference to epidermis may be ignored.

In the following genera, the pycnia are conical with flat basal hymenium and illustrated in the manual also: *Dasyspora* Berk. & Curt., *Cystomyces* H. & P. Syd., *Coleosporium* Léveille, and *Hyalopsora* Magn. But the pycnia are subepidermal and not subcuticular. Even so, *Hapalophragmiopsis* has subepidermal pycnia, conical, with flat basal hymenium, and is a genus distinct from *Hapalophragmium* with subcuticular pycnia.

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A NEW EMERICELLOPSIS SPECIES WITH STILBELLA-TYPE OF CONIDIA

P. N. Mathur and M. J. Thirumalachar

(with 7 figures)

Isolation of fungi from soil samples collected near Mount Abu, Rajasthan, India, included an *Emericellopsis* species which proved to be of considerable interest. On glucose-yeast extract agar, the fungus grew rapidly and produced numerous cleistothecia bearing ascospores characteristic of *Emericellopsis* van Beyma. In the centre of young colonies as well as at the margins of old ones, numerous white synnemata, 2–3 mm long, with a spherical head having glistening droplets of mucilage were present. The presence of a stilbaceous type of fruiting structure in species of *Emericellopsis* was peculiar and unknown. Consequently single ascospore cultures and single conidial isolations from the synnemata were made and pure cultures grown. The studies confirmed that the conidial stage observed in association was genetically connected and belonged to the life-cycle of the *Emericellopsis* species under study. All recorded species of *Emericellopsis* have only a *Cephalosporium* type of conidial stage, and hence the present species was different from any of the previously known species.

On glucose-yeast extract agar, the fungus made a slow, heaped-up whitish type of growth. Numerous furrows developed in the centre of the colony in a radiating manner. Very soon white aerial hyphae were formed as a felty mass covering the colony surface. After 15 days incubation at 24° C, the development of the conidial stage commenced. The initials of the synnemata appeared as button-shaped out-growths and rapidly elongated to form erect cylindrical structures. As the diameter of the colony enlarged, new synnemata were produced centrifugally.

Mature synnemata were 2 to 3 mm long, white, composed of cylindrical stipes and globular heads. The hyphae composing the stipe were parallel and closely interwoven. At the apex, the hyphae were parallel and functioned as conidiophores. These were simple and not branched, and developed acrogenously numerous one-celled, ovate-oblong, thin-walled conidia. These were hyaline and became embedded in a mucilaginous matrix. Due to the presence of numerous conidia in the muci-

laginous matrix, the tip of the synnemata appeared as a sphaeroidal structure. The conidia measured 3.5–6.5 × 2–3 μ and were readily dispersed in droplets of water. In structure, the conidial stage was identical with species of *Stilbella* Lindau. There was no conidial formation of the type similar to *Cephalosporium* either on the mycelium or synnemata.

After the initiation of conidial development, the cleistothecia began to form in large numbers. When mature, they imparted a greyish-white appearance to the colony. Mature ones measured 150–300 μ in diameter. They were subglobose to spherical, astomous, and completely covered by a hyphal pellicle. The wall of cleistothecium was 5–6-layered, inner layers being hyaline and outer ones coloured and thick-walled. The interior of the cleistothecium was filled with numerous asci and ascospores. The asci were hyaline, globoid 9–15 μ in diameter with thin walls, which were ephemeral and released the ascospores at maturity. The ascospores were 8 in number, brownish-black, and measured 5–6.5 × 3.5–4.5 μ. They were one-celled, ovate-elliptic to oblong, with 3 lateral wings, the flanges being 1.5–2 μ wide. The spores germinated readily and developed colonies producing the *Stilbella*-type of conidia.

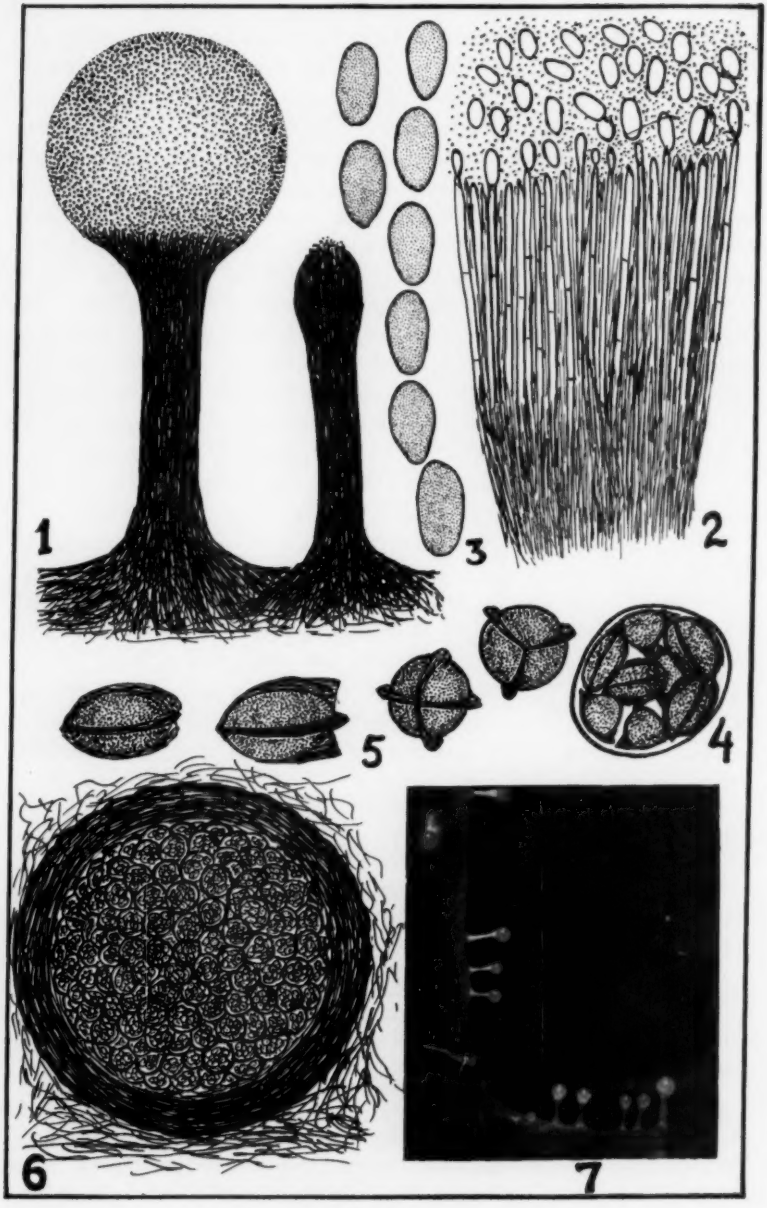
Owing to the presence of the *Stilbella*-type of conidial stage, the *Emericellopsis* species under study is different from all the other species. Considerable confusion has been brought in by Durrell (**1**) and Maag et al. (**2**) by unwarranted use of statistical and biochemical data in the taxonomy of *Emericellopsis*. This is being referred to in a separate paper. The name *Emericellopsis synnematicola* is proposed for the species under study (FIGS. 1–7). Unlike some of the other species of *Emericellopsis*, the present one has not shown any appreciable antibiotic activity against bacterial pathogens.

Emericellopsis synnematicola Mathur & Thirumalachar, sp. nov.

Cleistothecia glabra, globosa, 150–300 μ diameter. Paries cleistotheci, subhyalinus. Asci globosi vel subglobosi; 8-spori, evanescentes, 9–15 μ in diameter. Ascosporae ovate-ellipsoideae, flavo-brunneae, 1-cellulatae, 5–6.5 × 3.5–4.5 μ.

Status conidicus est *Stilbella;* synnematibus gregariis vel subsparsis, 2–3 mm long., albidis, rigidis, glabris, in capitula globoso-turbinata, hyphis stipatis conidiophoris filiformibus septulatis, hyalinis; conidiis acrogenis, copiossimis, ovato-ellipsoideis, 3.5–6.5 × 2–3 μ muco, primitus obvolutis.

Colonies on glucose-yeast extract agar, slow growing, white, with radial furrows, covered by white subaerial mycelium; colony reverse white at first and later becoming black after development of cleistothecia. Conidial stage of *Stilbella*-type; synnemata sparse or gregarious, white, 2–3 mm long, erect, with cylindrical stipe and globose head; stipe com-



FIGS. 1–7.

posed of closely interwoven hyphae, terminating with simple conidiophores bearing acrogenously numerous one-celled conidia held in mucous mass. Conidia ovate-elliptic, thin-walled, hyaline, smooth, 3.5–6.5 × 2–3 μ.

Ascigerous (*Emericellopsis*) stage developing as black cleistothecia, abundant, 150–300 μ in diameter, with numerous asci. Asci 9–15 μ in diameter, 8-spored, evanescent at maturity; ascospores yellowish-brown, one-celled, ovate-elliptic, 5–6.5 × 3.5–4.5 μ with 3 longitudinal wings, which are 1.5–2 μ in width.

Isolated from soil sample, Mount Abu, Rajasthan, India.

TYPE culture is deposited in Centraalbureau Voor Schimmelcultures, Baarn; in CMI, Kew, England and in the Division of Mycology, IARI, New Delhi.

SUMMARY

The description of *Emericellopsis synnematicola*, a new species with *Stilbella*-type of conidial stage, is given. All *Emericellopsis* species, previously known, have only the *Cephalosporium* type of conidia.

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LITERATURE CITED

1. **Durrell, L. W.** 1959. Some studies of *Emericellopsis*. Mycologia **51**: 31–43.
2. **Maag, G. W., L. W. Durrell, and M. G. Payne.** 1959. A chromatographic study of the fungus *Emericellopsis*. Bull. Torrey Bot. Club **86**: 120–125.

FIGS. 1–7. *Emericellopsis synnematicola*. 1. Synnemata bearing conidia, × 40. 2. Apex showing conidiophores, × 1000. 3. Conidia, × 2500. 4. Ascus, × 2000. 5. Ascospores, × 3000. 6. Cleistothecium with numerous asci, × 300. 7. Photograph showing *Stilbella*-type of synnemata growing from the margin of cut agar surface, × 5.

SOME LEAFSPOT FUNGI ON WESTERN GRAMINEAE–XIV [1]

RODERICK SPRAGUE [2]

(WITH 2 FIGURES)

Continued search in the western area has brought to light additional material contributing to our knowledge of the fungi parasitizing grasses. A number of collections were made June 10, 11, 1959 along French Creek, Snohomish County, Washington. In late July of the same year some specimens were obtained in the vicinity of Mt. Adams, Yakima County, Washington. Still later in August and early September, 1959 several hundred specimens were obtained in the Bitter Root Mountains along the Idaho-Montana border and then scatteringly eastward through Montana, North Dakota, Minnesota and Michigan.

Additional specimens were obtained by examination of the phanerogamic grass collections of the Department of Botany Herbarium, Washington State University, Marion Ownbey, Curator (**23**). In addition, a number of specimens were sent in for determination. The first mentioned species in this paper is included in this category.

Stagonospora sporobolicola Sprague, sp. nov.

Maculis nullis; pycnidiis dispersis, nigris, globosis, ostiolatis, erumpentibus, 70–110 μ diam.; pycnidiosporulis hyalinis, triseptatis, cylindraceis, utrinque rotundatis, 25–30 × 6.0–6.8 μ.

Spots none; pycnidia scattered, but not scarce, black, globose, erumpent, slightly pointed toward the ostiolar area, 70–110 μ diam; spores hyaline, triseptate, cylindrical or sometimes with a distal cell larger than the others, both ends rounded, 25–30 × 6.0–6.8 μ.

Hab. in dead leaves of *Sporobolus poiretii* (Roem. & Schult.) Hitchc., leg. *L. M. Pedersen Corrientes,* Depto. Mburucuyá, Est. Santa Teresa, Argentina, July 9, 1956. The TYPE is Universidad Nacional De La Plata Museo Instituto Spegazzini Colecciones micologicas No. 28039 sent

[1] Scientific Paper No. 2033, Washington Agricultural Experiment Stations, Pullman. Project No. 449.

[2] Pathologist, Washington State University, Tree Fruit Experiment Station, Wenatchee.

by courtesy of Curator J. C. Lindquist. The type has been returned to Dr. Lindquist but one slide and a fragment of the holotype is filed in the Plant Pathology Herbarium, Washington State University, Pullman as WSP 46446.

St. sporobolicola is similar to *St. tridentatis* Sprague & Rogerson (**30**) but the spores are somewhat larger. It is another of the many saprophytic or weakly parasitic species of *Stagonospora* that occurs on grasses, sedges and associated monocotyledons. After detailed comparison I decided that the nearly cylindrical shape of the spores of *St. sporobolicola* excluded it from the *St. subseriata* complex. The spores are also too narrow for *St. simplicior* Sacc. & Berl. and too small for *St. vexatula* Sacc. It is closer to *St. typhoidearum* (Desm.) Sacc. (cf **15**, v. 3, p. 451) and in this group perhaps nearest to *St. typhoidearum* f. *sparganii* Fckl. This, however, like many others, is described as having fusiform spores and this is not typical of Pedersen's collection (FIG. 2, A).

Phyllosticta parvimammata Sprague, sp. nov.

Maculis striatis, griseis, centro pallido, margine obscuris vel tarde griseis, 4–5 × 0.3–0.7 mm diam.; pycnidiis aureo-brunneis, globosis, in lineis dispositis, erumpentibus, ostiolatis, minutis, 40–85 μ diam.; pycnidiosporulis minutis, hyalinis, bacillaribus, aseptatis, 4–6 × 0.5–0.9 μ, rectis vel subcurvulis.

Lesions striate, gray, becoming pale in center, indefinite-margined or tardily gray, 4–5 × 0.3–0.7 mm diam; pycnidia golden brown, globose, obscure, disposed in lines, erumpent, obscurely ostiolate, small, 40–85 μ diam; pycnidiospores minute, hyaline, bacteria-like, aseptate, 4–6 × 0.5–0.9 μ, straight or bent, sometimes somewhat comma shape.

Hab. on dried sheaths of *Calamagrostis langsdorfii* (Link) Trin., Distr. of Tomsk, Siberia, USSR, leg. *L. Sergievaskaja*, Aug. 9, 1925. TYPE is WSP 46356 ex WS 102085.

This is probably the microspore stage of a fungus with larger macrospores. The microspores of *Septoria avenae* Frank found at Priest Lake, Idaho were 4–5 × 1.1–1.5 μ, somewhat wider than the ones from Siberia. A collection of a similar fungus on *Glyceria striata* (Lam.) Hitchc. with tiny pycnidia, 30–40 μ diam, and microspores 6.8–7.6 × 0.8 μ was also tentatively assigned to *S. avenae* (**26**). *P. parvimammata* has symptoms somewhat distinct from those of *S. avenae* and the microspores, as indicated, are smaller. The symptoms and the arrangement of the pycnidia are more like *Selenophoma everhartii* (Sacc. & Syd.) Sprague & A. G. Johnson but I have no information on microspores in this genus. One

therefore has the choice of naming this material or ignoring it. Since it comes from a region that probably has not been fully explored, it seemed desirable to give it a name as it may very well be part of the life cycle of a fungus not known to North America.

Examination of leaves of winter rye (*Secale cereale* L.) at the W.S.U. Vancouver Experiment Station, Wash., disclosed a scattering of elliptical to circular pale leafspots bearing relatively prominent fruiting bodies. Part of the plots had been heavily sprayed with 2,4-D weed killer and had suffered severe shock from the treatment. The prevalence of somewhat parasitic fungi on this usually healthy cereal indicates that the chemical may have predisposed the rye plants to attack. In my first examination I found ASCOCHYTA HORDEI Hara with yellowish spores 17–23 × 5–6.5 μ associated with a species of *Leptosphaeria* with 3-septate fusiform spores (WSP 46415). Two other mounts showed only traces of the *Ascochyta* but an abundance of the *Leptosphaeria* associated with subhyaline spores of *Phaeoseptoria festucae* Sprague. The *Leptosphaeria* in both instances had spores 22–28 × 6–7 μ and is referable to L. MICROSCOPICA Karst. Webster (**33**) in carefully detailed work has indicated that *P. festucae* is a phase of *L. microscopica*. To the confusing array of fungi in these spots on this material may be added a species of *Phyllosticta* with spores 4.5–5.5 × 2.0–2.7 μ. This is close to *P. sorghina* Sacc. except that the spores are proportionately broader in relation to their length than most collections of *P. sorghina* which, incidentally, occur on warm-temperature hosts.

The above mentioned *Leptosphaeria* was also compared with *L. herpotrichoides* de Not. which Mary D. Glynne, G. W. Bruehl and I found nearby on basal sheaths of rye, also apparently treated with an overdose of 2,4-D (WSP 46510). *L. herpotrichoides* has 5–7-septate spores, mostly 28–30 × 4.0–4.2 μ in our material (WSP 46417) and is readily distinguished from *L. microscopica*. *L. secalis* Haberl., from its description, appears to be a synonym of *L. herpotrichoides* and need not be further considered here (**15**, v. 2, p. 76).

ASCOCHYTA AVENAE (Petrak) Sprague & A. G. Johnson was relatively common on red oats (*Avena byzantina* K. Koch) (WSP 46418) and common oats (*Avena sativa* L.) (WSP 46434, 46553, 46554) in yellow dwarf virus plots at W.S.U. Experiment Station, Vancouver, Washington. The prominent pycnidia were in elliptical, white spots with red-brown borders or in discolored areas without prominent borders. The virus-injured plants appeared to be abnormally susceptible to this usually inconspicuous species of *Ascochyta*.

Ascochyta hordei was also found on *Hordeum vulgare* L. at Colum-

bus, Ohio, by C. W. Ellett, April 18, 1959. The irregular lesions had a strongly marked brown border with a pale gray to isabelline center. Most of the lesions occurred from 1.5 to 2 inches from the leaf tip (FIG. 1, A). Several lesions were partially coalesced or gregariously arranged to cover the entire width of the leaves at certain points. The yellowish spores were typical of *A. hordei* from the western United States (**29**, Fig. 2, G). The original slide mount of spores and pycnidia from Ellett's collection is filed at Pullman (WSP 46366). It should be mentioned that *A. hordei* was also found on living leaves of winter wheat (*Triticum aestivum* L.) 5.2 miles east of Davenport, Washington, February 13, 1960, after the snow had melted. Definite circular, tawny to gray lesions were sparingly scattered over the leaves, sometimes in the center of the leaves. The fungus appeared to be actively parasitic at the recently prevailing temperatures of about 40–50° F daytime and 20–30° F night time. Spores were at first hyaline, 14–20 × 3–4 μ, but when mature were typically yellowish, 14–22 × 4.6–7.5 μ (cf **20**, Fig. 10) in pycnidia as large as 175 μ diam. The arrangement of the pycnidia tended to give the eyespots a ring spot aspect (cf **20**, p. 156). This is a newly reported host for the region. In an earlier discussion of *A. hordei* (**20**) I had mentioned *A. tritici* Hori & Enjoji which has spores 14–21 × 3–6.5 μ borne in spots free of rings. The material from Washington resembles *A. tritici* in its active stage in young spots but the older material (intermingled with *Cladosporium* and *Heterosporium*) at the tip of the leaves has yellow spores typical of *A. hordei*. There are intermediate stages present. It is indicated that *A. tritici* is a phase of *A. hordei*. While the saprophytic material was fairly abundant in our collection, the round spots surrounded by live tissue were not common. Later I found leaves of a Chinese wheat variety (spring type) in varietal plots at the Southwestern Washington Experiment Station, Vancouver, Washington, February 16, 1960, showing lesions of *A. hordei*. While most of the lesions in this collection were caused by *Septoria tritici* Rob. in Desm. (WSP 46524), the *Ascochyta* spots were distinguishable by their brown border (WSP 46523). One leaf of this collection showed vague target spotting.

Phyllosticta glynnea Sprague, sp. nov.

Maculis flavidis, elongatis, margine brunneis; pycnidiis prominulis, aureo-brunneis, globosis, ostiolatis, pseudoparenchymatosis, 70–145 × 70–140 μ; pycnidiosporulis hyalinis, ellipsoideis vel ovoideis vel subphaseoliformibus, 4–7 × 2.5–3.3 μ.

Spots yellowish to buff, elongate, margin brown, well defined; pycnidia thin-walled, prominent, yellow brown, globose, erumpent with

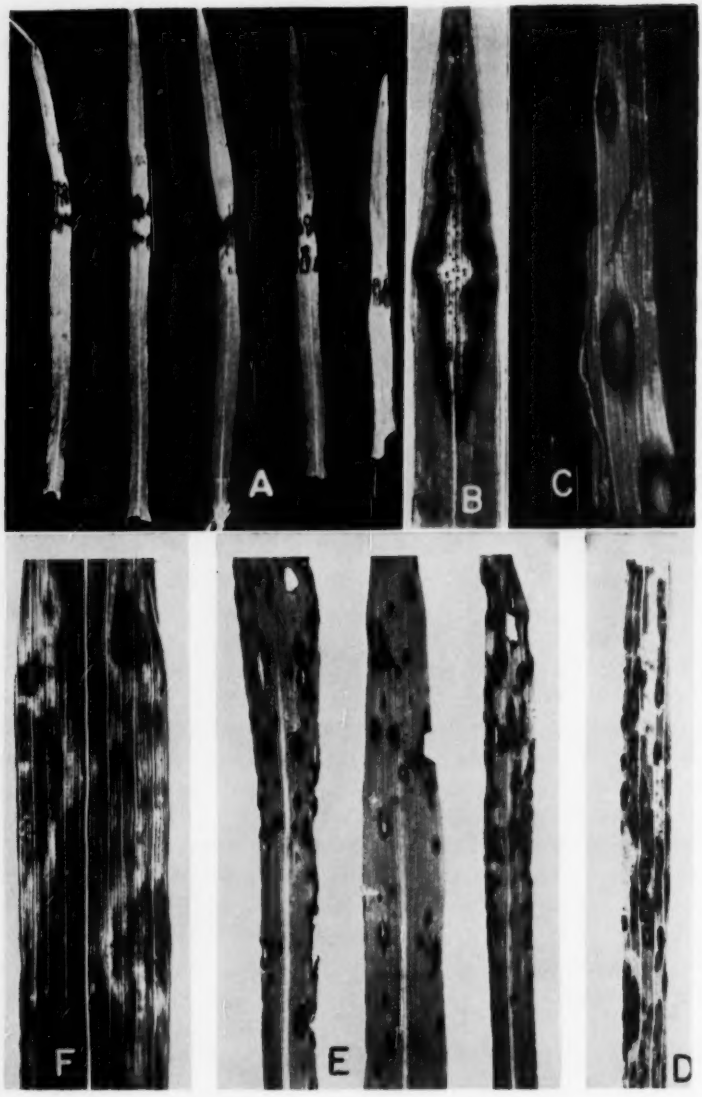

FIG. 1, A–F.

FIG. 1. Leafspot fungi. A. *Ascochyta hordei* on *Hordeum vulgare*, Columbus, Ohio (WSP 46366). × ⅔. B. *Septoria avenae* on *Glyceria pauciflora*, Loup Creek near Ward Creek, St. Joe, N. For., Ida. (WSP 46475). × 3. C. *Drechslera tu-*

small ostioles, pseudoparenchymatous to almost membranaceous; spores exuded in long thick tendrils, bright hyaline, ellipsoid, ovoid or often somewhat phaseoliform, nonseptate, 4–7 × 2.5–3.3 μ.

Hab. on living leaves of *Poa annua* L. growing as a roadside weed on the Southwestern Washington Experiment Station, Vancouver, Washington, June 26, 1959, leg. *R. Sprague*, WSP 46435 (TYPE). The fungus is named for Mary D. Glynne, Pathologist, who was a visitor at the station on the day the specimen was collected.

This fungus has spores too small for any species of *Macrophoma* known on Gramineae in the Northern Hemisphere. The spores are too irregular and the pycnidia differ from those of *P. sorghina*. The Vancouver collection is close to *P. kerguelensis* P. Henn. (**15**, v. 22, p. 865), especially in spore size. The southern (Antarctic) fungus, however, has dark, innate pycnidia which are subglobose, lenticular and only 60–80 μ diameter. Because of this distinct difference the specimen from Vancouver is a recognizable species.

It should be mentioned that the host had been heavily sprayed with 2,4-D weed sprayer which may have predisposed the grass to this previously unreported leafspot. I was surprised when I examined the material that it was not the common *Septoria macropoda* Pass. A glass slide of the original mount is filed in the type packet.

STAGONOSPORA BROMI Sacc. occurs in typical dark lesions on a few leaves of *Bromus carinatus* Hook. & Arn. collected near Vancouver, Washington (WSP 46462). Of interest is the fact that *Phyllosticta* sp. is associated with some of the material. The *Phyllosticta* has pale brown, globose, erumpent pycnidia, 60–115 × 60–90 μ and contains mostly elliptical to obovate hyaline spores 5.3–7.5 × 3.0–3.5 μ. Except that the spores are smoky in mass and are proportionately shorter (FIG. 2, C) than those of the type of *P. bromivora* Sprague which has spores 6–11 × 3.0–3.8 μ (**21**, Fig. 1, C), the *Phyllosticta* from Vancouver could be assigned to *P. bromivora*. I believe, however, that the associated *Phyllosticta* and *St. bromi* from Vancouver are stages of the same species. The pycnidia are comparable, the spore masses each show a smoky tint, the spores have the same hyaline or faintly chlorine aspect and are about the same width. Nonseptate spores identical to the *Phyllosticta* occur in pycnidial exudates with a preponderance of sep-

berosa on *Cynosurus echinatus*, Mill Valley, California (WSP 46329 ex WS 103082). × 3. D. *Heterosporium phlei* on *Phleum pratense*, Mineral Creek, Ida. (WSP 46467). × 2. E. *Piricularia grisea* on *Pennisetum purpureum*, Panama (WSP 46589). × ⅔. F. *Colletotrichum graminicola* on *Phalaris arundinacea*, Riffe, Wash. (WSP 46496). × 2.

tate spores from some pycnidia. We have kept one leaf separated as WSP 46461 because it has considerable of the *Phyllosticta* phase while WSP 46462 from the same field collection is mostly *St. bromi.* Earlier when we described *P. bromivora* we found no evidence of any relation with a large spored species. It must be retained as a separate species on present evidence. Its pseudo-facsimile from Vancouver is considered

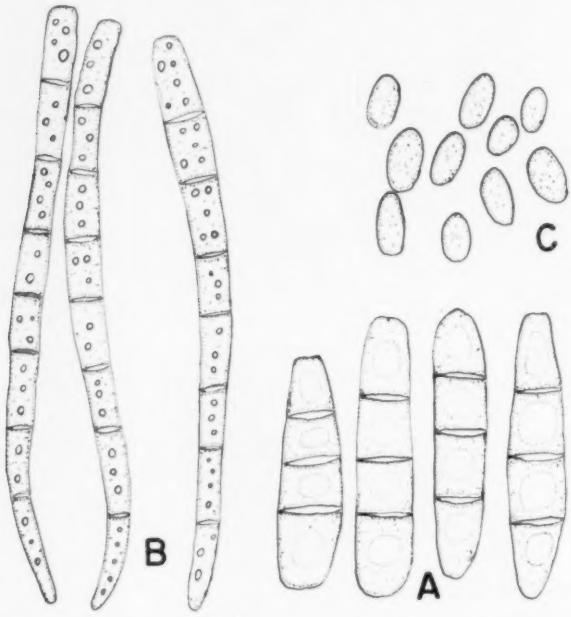

FIG. 2. Pycnidiospores. × 667. A. *Stagonospora sporobolicola,* type. B. *Phaeoseptoria festucae* var. *muhlenbergiae* on *Triticum aestivum,* near Ethel, Wash. C. *Phyllosticta* phase of *Stagonospora bromi* on *Bromus carinatus,* Vancouver, Wash.

distinct from *P. bromivora* and only a developing phase of *Stagonospora bromi.* I also found *Phyllosticta* sp. on necrotic leaves of spring barley which was almost killed by 2,4-D, *Ophiobolus graminis* Sacc. and probably yellow dwarf at the Vancouver Experiment Station. The spores were smoky, 4.0–6.5 × 2.2–3.2 μ, in brown pycnidia, 80–114 μ diam (WSP 46464).

Scanty material of *St. bromi* was collected on *Bromus vulgaris* (Hook.) Shear in woods near the top of Skinner's Butte, Eugene, Oregon, June 15, 1960 (WSP 46574).

STAGONOSPORA ARENARIA Sacc. occurred in tawny lesions on leaves of *Dactylis glomerata* L. at Montreal, Quebec Prov., Canada, August 14, 1959 (WSP 46473). The pycnidiospores of this collection are too large for *Septoria avenae* and not broad enough for *Stagonospora maculata* (Grove) Sprague. As earlier noted (**19**), I consider it necessary to include this type of material on *Dactylis* under *St. arenaria*.

Scattered pycnidia of STAGONOSPORA GLYCERICOLA Sprague occurred on dried leaves of *Glyceria pallida* (Torr.) Trin. near Laurier, Quebec, August 22, 1959 (WSP 46479). The spores were typical of those in the type (**18**, Fig. 2, E). The same fungus on *G. grandis* S. Wats. was found at Bonfield, Ontario, August 14, 1959 (WSP 46498). The known range of this fungus now extends through eastern North Dakota, Minnesota, Ontario to southcentral Quebec.

STAGONOSPORA FOLIICOLA (Bres.) Bubak was found in buff spots on *Phalaris arundinacea* L. at Ajlune, Lewis County, Washington, November 5, 1959 (WSP 46488, 46489). An immature ascomycete was present on recently dead leaf parts in this material also.

Some fragmentary material of a spot on leaves of *Calamagrostis inexpansa* A. Gray contains pycnidia with chlorine-tinted *Ascochyta* spores measuring 27–34 × 10–12 μ. This material was collected by W. K. W. Baldwin (2477) at Reindeer Lake, Manitoba, August 14, 1951 (WSP 46413 ex WS 213938). The spores are too large for *A. avenae*. The fungus is more likely the summer phase of STAGONOSPORA SIMPLICIOR Sacc. & Berl. which is not uncommon on sedges in habitats similar to that occupied by *C. inexpansa*. Associated are some immature, clavulate, tinted spores, possibly *Phaeoseptoria*. Additional and better material of the *Calamagrostis* spot is needed.

SEPTORIA BROMI Sacc. was found on leaves of *Bromus anomalus* Rupr. collected by C. G. Pringle, September 18, 1894 at 9500 feet elevation in the Sierra de San Felipe, Oaxaca, Mexico (WSP 46342 ex WS 2701). The grass was labeled "near *purgans*" but in a modern key traces readily to *B. anomalus*. We have no report in our records of *S. bromi* from Mexico (**19**, **28**) and this is the first instance of *S. bromi* on *B. anomalus*. *S. bromi* was also found on dried leaves of *Bromus commutatus* Schrad. at Ajlune, Lewis County, Washington, June 28, 1959 (WSP 46443).

SEPTORIA TENELLA Cooke & Ellis occurred on scattered ashy spots of the woodland grass, *Festuca subuliflora* Scribn., growing at the edge of woods along French Creek (WSP 46376). The spores are 0–1-septate and identical, except for more prevalent septations, with some spores illustrated recently on *F. subulata* Trin. (**25**, Fig. 1, A). One

pustule of *Puccinia coronata* Corda was also seen on *F. subuliflora*. Both fungi are unreported on this host which appears to be resistant to most fungi. The host is not included in earlier literature (**19**, **28**) as these are apparently the first reports of parasitic fungi on this grass.

SEPTORIA AVENAE Frank was prevalent on *Agrostis tenuis* var. *aristata* (Parnell) Druce at Campbell River, B. C., August 21, 1960 (WSP 46615). Some material from Oregon on *A. hallii* Vasey and on *A. scabra* Willd. from North Dakota which was assigned to *S. secalis* var. *stipae* Sprague (cf **20**, p. 254) should now be segregated from the collections on *Stipa* and transferred to *S. avenae* as continued study indicates that *S. avenae* is the common cylindrically spored *Septoria* on *Agrostis*.

Septoria avenae was common on *Glyceria canadensis* (Michx.) Trin. near Sowerby, Ontario in August, 1959 (WSP 46490). A typical lesion of this fungus on *Glyceria* is shown in FIG. 1, B.

SEPTORIA AVENAE f. sp. TRITICEA T. Johnson caused elongated buff lesions on leaves of *Agropyron trachycaulum* (Lk.) Malte along a stream in a canyon east of Townsend, Montana, September 4, 1959 (WSP 46515). The cylindrical spores were 1–3-septate, hyaline, 30–45 × 2.9–3.2 μ, borne in scattered pycnidia. The collection seems to fit the above mentioned fungus. It is sometimes found on *Elymus* spp. and also occasionally on the closely related genus *Agropyron*. It has been found on *A. smithii* Rydb. in Montana and on *A. trachycaulum* in North Dakota (**20**, p. 223). Some material on *E. mollis* Trin. collected along the shore at Qualicum Bay near Bowser, B. C., appears to belong here. The specimen (WSP 46610) was removed from a larger collection of *Puccinia recondita* Rob. (WSP 46609). The pycnidia occur in elongate, buff lesions. The spores were immature, mostly 1-septate, but a few were tri-septate, cylindrical, hyaline, 19–28 × 2.8–3.2 μ mostly 22 × 3.0 μ.

SEPTORIA MACROPODA Pass., a common leafspot on *Poa annua*, was noted at St. Adele, Quebec, August 18, 1959 (WSP 46505). S. OUDEMANSII Sacc., also common, occurred on *Poa pratensis* L. at Montreal and vicinity (WSP 46471).

SEPTORIA POLIOMELA Sydow occurred on basal sheaths of the annual grass *Aira caryophyllea* L. in the grassy hills of Blanton Hts., Eugene, Oregon, collected June 16, 1960. The scattered brown pycnidia were 60–115 μ diam containing immature narrowly cylindrical spores 8–16 × 1.8–2.2 μ, mostly 0–1-septate. *S. poliomela* is not rare on *Deschampsia* (**20**, **27**) in the western United States but this is the first material

that I have seen on the type host since examining the type from the Canary Islands (cf **31**).

SEPTORIA CALAMAGROSTIDIS (Lib.) Sacc. occurred on *Agrostis scabra* growing in sterile soil at Lake Roberts, Vancouver Island, B. C. (WSP 46606), August 21, 1960. The filiform curved spores were 35–56 × 1.0–1.5 μ.

A few spores of SELENOPHOMA EVERHARTII (Sacc. & Syd.) Sprague & A. G. Johnson were found on a moldy fragment of an old leaf of *Calamagrostis purpurascens* var. *maltei* Polunin collected by M. O. Malte on Baffin Island, Canada, August 15, 1927 (WSP 46353 ex WS 223992). The spores ranged to as small as 7 × 1 μ. There is no report of *Selenophoma* on this grass and no previous report, as far as I can determine, of a leafspot on any grass from this isolated area. Baffin Island lies northeast of Hudson Bay in the District of Franklin of Northwest Territories. *S. everhartii* also occurred in elongated spots on leaves of *Calamagrostis koelerioides* Vasey along the south shore of Upper Canyon Lake, eleven miles north of Dedrick, California at 6200 feet elevation. The specimen was collected by C. L. Hitchock and J. S. Martin, July 11, 1935 (WSP 46402 ex WS 95795).

SELENOPHOMA DONACIS (Pass.) Sprague & A. G. Johnson was common on heads and upper portions of the culms of dried plants of *Agropyron elongatum* (Host.) Beauv. collected near the community of Mold in the winter wheat area of Douglas County, Washington, September 29, 1959 (WSP 46482). The presence of pycnidia of this fungus in such a dry area is probably explained by the fact that precipitation during the last three weeks in September was 1.91 inches compared with an average for September of 0.61 inches. There has been no previous report of *Selenophoma* on *A. elongatum*. Since this grass has been used in crossing with *Triticum aestivum* to obtain a possible perennial wheat, any known parasites on the grass are of interest. *S. donacis* was also common on scattered plants of *A. desertorum* (Fisch.) Schult. (WSP 46483) in the field at Mold. This crested wheatgrass appears to be resistant to *Selenophoma*. Some well formed eyespots of *S. donacis* were found on *Poa palustris* L. at Riviere a la Marte, Gaspé Peninsula, Prov. Quebec by W. B. and V. G. Cooke (31546), August 13, 1959 (WSP 46546). *S. donacis* was also found on *Aegilops cylindrica* Host. growing as an escape near Union Flat Creek, Whitman County, Washington, July 7, 1960 (WSP 46580). The spores (14–21 × 2.6–3.0 μ) were found in tiny black pycnidia (60–88 μ diam) scattered along the silicified awns. I have no report of *Selenophoma* on *Aegilops*.

Bromus carinatus, in its wild state, at least, is moderately resistant

to SELENOPHOMA BROMIGENA (Sacc.) Sprague & A. G. Johnson. I recently found relatively abundant material, however, near the spillway of Bonneville Dam, Washington (WSP 46447). Here the air was saturated with spray and conditions were ideal for the fungus. The lower leaves of plants of *B. carinatus* were almost covered with pycnidia while typical eyespots extended to the top leaves of the plants. The heads also carried smaller fruiting pycnidia.

SELENOPHOMA DONACIS var. STOMATICOLA (Bauml.) Sprague & A. G. Johnson occurred on dried culms of *Danthonia spicata* (L.) Beauv. collected near Hawkesbury, Ontario, August 14, 1959 (WSP 46478). The spores are strongly curved, small, 12–16 × 1.6–2.4 μ. This appears to be summer material of var. *stomaticola,* group one (cf **20**, p. 207, 208). The same fungus was found on this grass at St. Theodore, Quebec, August 18, 1959 (WSP 46494). The fungus is also omnipresent as a stem speckle on dead, overwintering culms of *Puccinellia airoides* (Nutt.) Wats. & Coult. collected near Molson, Washington (WSP 46506), December 16, 1959. The grass had been growing in white clay with pH 8.5.

S. donacis var. *stomaticola* produced numerous coalesced to extensive spots on leaves of *Sporobolus cryptandrus* (Torr.) A. Gray growing at the edge of an orchard on the banks of the Columbia River near Brewster, Washington on July 26, 1960. The grass had been under sprinkler irrigation for 12-hour periods at bi-monthly intervals since April. While infection was heavy, pycnidial production was very scanty. The heat-inhibited spores were somewhat small, mostly 12–15 × 1.9–3.0 μ (WSP 46585).

A specimen sent by H. C. Greene showing light colored circular to elliptical spots with reddish borders occurs on *Sporobolus asper* (Michx.) Kunth. collected near Cassville, Wisconsin, June 23, 1959. The 3-septate clavulate spores are brown in mass but hyaline or faintly tinted when separated. They measure 46–68 × 3.3–4.1 μ. I refer these to SEPTORIA ANDROPOGONIS J. J. Davis rather than *Phaeoseptoria* because of the evident parasitic nature of the fungus plus the fact that the spores are as hyaline as some well-recognized species of *Septoria.*

PHAEOSEPTORIA FESTUCAE var. MUHLENBERGIAE Sprague, or a facsimile of it, occurs scatteringly on dead leaf parts of *Triticum aestivum* collected near Ethel, Lewis County, Washington, June 28, 1959 (WSP 46450). The leaves had also been invaded by *Septoria tritici* and leaf rust. The large pycnidia of the *Phaeoseptoria* contain masses of clavulate-filiform, light yellow spores, 50–81 × 4.0–5.2 μ, faintly 7-septate (FIG. 2, B). This fungus should be compared critically with some of

the coarse spored species described as *Septoria* on *Triticum* such as *S. triticina* Lobik.

MACROPHOMA SPOROBOLI Sprague produced a few scattered pycnidia of *Oryzopsis hymenoides* (Roem. & Schult.) Rick. on dried, overwintered leaves in the desert near Tonopah, Nevada (WSP 46458). Most of the material was infected with *Puccinia burnettii* Griff. (WSP 46457), but one leaf contained a few pycnidia with obovate spores, 14–15 × 5.5–6.0 μ. This fungus seems to be restricted to the drier desert habitats.

HENDERSONIA CULMICOLA Sacc. occurs on necrotic leaves of *Bromus ciliatus* L. collected at Solway, Minnesota, August 11, 1959 (WSP 46621). The yellow, cylindrical spores measured 31–36 × 3.3–4.0 μ.

COLLETOTRICHUM GRAMINICOLA (Ces.) Wils. caused a mold on leaves of *Cynodon dactylon* (L.) Pers. in Edwards County, Texas (WSP 46346 ex WS 168484). The fungus was also obtained on *Calamagrostis neglecta* (Ehr.) Gaertn., Mey. & Schreb. at Lake Minnetonka, Minnesota, by E. T. Harper, July 7, 1888 (WSP 46355 ex WS 58568). Although this phanerogamic specimen was obtained over seventy years ago, the grass has apparently never been reported as harboring the common mold, *C. graminicola*. The fungus was also found on *C. canadensis* (Michx.) Beauv. at Estherville, Iowa, August 10, 1909 by B. Shimek (WSP 46409 ex WS 184231) and on the same grass collected at Bruce Mines, Ontario (WSP 46481), a frequent host. The same fungus caused black striae on leaves of *Ammophila breviligulata* Fernald growing in dune sand along the shore of Lake Superior near Marquette, Michigan, August 12, 1959 (WSP 46474). The fungus was also common on basal leaves of *Festuca dertonensis* (All.) Aschers. & Graebn. at French Creek, Snohomish County, Washington (WSP 46371). *C. graminicola* also developed in late fall (Nov. 5, 1959) on *Phalaris arundinacea* at Riffe, Lewis County, Washington (FIG. 1, F). It caused large yellowish to buff lesions on green leaves. The tiny black acervuli were numerous on some of the lesions. This host is apparently an uncommon one for this fungus, invasion coming during late season. The same fungus is also common on *Dactylis glomerata* in the northeastern United States. I found it near Central Square, New York, August 29, 1959 (WSP 46497). I also found *C. graminicola* on the perennial leaves of *Hierochloë odorata* (L.) Beauv., collected at Santa Claus Village, Val Morin, Quebec, August 18, 1959 (WSP 46517).

RHYNCHOSPORINA MEINERSII (Sprague) V. Arx caused light buff streaks on living leaves of *Phleum pratense* L. in the fields near Rockland, Ontario, August 14, 1959 (WSP 46500). A few areas on the

lesions have shallow white masses of spores. The spores clung together for some time in water mounts. They had the typical size and shape of the type of *Gloeosporium meinersii* Sprague, 10–14 × 3–4 μ. The material from Ontario was a confusing mixture of the *Rhynchosporina, Heterosporium phlei* Gregory (mostly sterile), and a mold on the older leaves which contained a few spores of DRECHSLERA PHLEI (Graham) Shoemaker (**3**, **16**). Most of the collection was bulked under WSP 46501. In another collection of *H. phlei* from Idaho, good material for illustrating symptoms was obtained (FIG. 1, D).

FUSARIUM NIVALE (Fr.) Ces. was isolated from a severe pink leaf and sheath mold on *Puccinellia distans* (L.) Parl. near Malo, Ferry County, Washington on April 19, 1960.

SCOLECOTRICHUM GRAMINIS Fckl. caused a white, red-bordered eyespot on *Cynosurus cristatus* L. near Seabeck, Washington (WSP 46331 ex WS 69248), collected by Louis A. Dillon 384, June 16, 1934. The symptoms in comparison with the elongated streak lesions usually caused by this fungus on other hosts are unusual and unexpected. The spores and conidiophores are typical of *S. graminis*. *C. cristatus* has been reported as a host of *S. graminis* from British Columbia and New York (**20**). *S. graminis* with typical streak symptoms was found on several herbarium specimens of *Bromus vulgaris* (Hook.) Shear as follows: Friday Harbor, Washington (WSP 46340 ex WS 65001); Glacier Park, July 9, 1941, leg. *Susan Fry* (WSP 46341 ex WS 108404); Bonner County, Idaho, leg. *R. F. Daubenmire* (WSP 46342 ex WS 181563); and Hubbards Station, Humboldt County, Washington in 1899, leg. *Davy* and *Blasdale* (WSP 46343 ex WS 30444). *S. graminis* occurred on basal leaves of *Agrostis variabilis* Rydb. at Midway Guard Station, Yakima County, Washington (WSP 46592) and nearby on Potato Hill on *Agrostis scabra* var. *geminata* (Trin.) Swallen (WSP 46597).

CERCOSPORELLA POAGENA Sprague, which has been found scatteringly in Oregon (**20**), southeastern Alaska (**22**) and in the Olympic Peninsula, Washington (**24**) was abundant and destructive to *Poa pratensis* in small clearings along French Creek and nearby on the banks of the Stillaguamish River, Snohomish County, Washington, June 11, 1959 (WSP 46372, 46375). The fungus produced many spores in eyespots and in vaguer areas where numerous lesions had coalesced to form blotchy areas. Such symptoms had been noted earlier at Hoonah, Alaska (**22**). This fungus is potentially troublesome on this economic host.

A few spores MASTIGOSPORIUM RUBRICOSUM (Dearn. & Barth.) Nannfeldt were seen in a mount from *Calamagrostis deschampsioides* Trin.

collected by W. J. Eyerdam at Port San Juan, Evans Is., Alaska (WSP 46408 ex WS 227584). There is no report of a parasitic fungus on this grass from Alaska. *M. rubricosum* was also found on a herbarium sheet specimen of *C. inexpansa* collected August 21, 1951 by Dan Lynch near Babb Jc., Glacier County, Montana (WSP 46357 ex WS 205938). The lesions are well developed and give no indication of host resistance although this appears to be the first report of this fungus on this grass. W. B. and V. G. Cooke collected *M. rubricosum* on the ubiquitous host *Dactylis glomerata* on Bonaventure Is., Gaspé Peninsula, Quebec (WSP 46549).

Some very scanty material on basal leaves of *Agrostis idahoensis* Nash collected at Spring Creek Forest Camp, T 10 N, R 10, E, Sec. 28, Yakima County, Washington, July 31, 1959, yielded a few spores of *Mastigosporium rubricosum* (WSP 46595). This is the first leafspot material which we had found on this host. However, a short time later we encountered a buff leafspot on this grass bearing an abundance of spores of *Rhynchosporium orthosporum* Caldwell. The latter specimen was collected above Muddy Meadow toward the base of Mt. Adams, Washington (WSP 46661).

Some excellent material of PIRICULARIA GRISEA (Cke.) Sacc. on *Pennisetum purpureum* Schumach was sent from Panama by Juan B. Ferrer in December, 1959 (WSP 46589). Neither Ferrer nor I can locate any reference to this grass as a host for *Piricularia* although from the lush development on the Panama material, it may not be uncommon (FIG. 1, E).

Ferrer also sent material of a white mold or honey dew on spikelets of *Hyparrhenia rufa* (Nees) Stapf. Some of the spore masses of the mold are somewhat sugary but on the edge the spore masses appear white and almost crystalline. One or two of the masses were honey yellow color and hard, perhaps forming sclerotia. The astringent sweet tasting spore masses are bright crystal hyaline, consisting of one celled, biguttulate, broadly cylindrical spores 14–22 × 6–8 μ. This may be the spermatial stage of a *Claviceps* sp. (WSP 46590). Further search for sclerotia is needed.

Recently Shoemaker (**16**) following work by Hughes (**5**, **6**) pointed out the unhappy status of the group of graminicolous fungi generally known as *Helminthosporium* (cf **2**). The original spelling of the genus as given by Link in 1809 was *Helmisporium* (**10**). The name was validated by S. F. Gray in 1822 (**4**). Link later changed the spelling to *Helminthosporium* (**11**) although *Helmisporium* Lk. ex S. F. Gray is legitimate (cf **8**, Art. 73). Hughes (**5**) pointed out that *H. velutinum*

Lk. ex S. F. Gray forms conidia both apically and laterally while the graminicolous species form conidia only at the conidiophore apex and produce a new apex by subterminal growth. Hughes later (**6**) retained only a few species in *Helmisporium* and excluded the graminicolous species, among others. Shoemaker (**16**) has taken Nisikado's subgenera *Cylindro-Helmisporium* and *Eu-Helmisporium* (**12**) and raised them to genus status accepting the first as *Drechslera* Ito (**7**) and creating a new genus *Bipolaris* Shoemaker to accommodate the subgenus *Eu-Helmisporium*. While some species of *Helmisporium* on Gramineae do not fit into the pattern too well, in general Shoemaker's classification seems to be practical.

Before I became aware that Dr. Shoemaker was studying the group, I had given up waiting for critical revisions to appear. I described three species and submitted two of these for publication. These were described as *Helminthosporium* but fortunately Shoemaker's paper appeared in time for me to append a footnote placing these in *Bipolaris*. One of these *B. arizonica* (**26**), is acceptable to Dr. Shoemaker (personal letter) but *B. gastridii* he believes may be close enough to *B. brizae* (Nisikado) Shoemaker to be included in that species. A specimen on *Briza minor* L. which I had labeled *H.* sp. he says is *B. brizae* (WSP 37353).

A fungus on *Cynosurus* which I tentatively called *Drechslera cynosuri* (in herb.) Shoemaker believes is *D. tuberosa* (Atk.) Shoemaker (**16**; **15**, v. 14, p. 1086–87). The fungus on *Cynosurus echinatus* L. from Mill Valley, Marin County, California (WSP 46329 ex WS 103082) shows straw-color to buff, elliptical spots with sharp pointed ends (eye-spots) (FIG. 1, C) with prominent yellow-brown margins. The material has brown conidiophores, straight, sometimes geniculated, 3–10-septate, 50–150 × 8–12 μ and scattered or rarely in groups of three. The conidia are yellow, the walls pale but prominent, spores elongate, cylindrical but tending to widen toward the base, hence somewhat club-shaped, 60–90 × 12–15 μ and 1–7-septate with a hilum scar present but scarcely exposed. I had earlier reported a collection from Gold Beach, Oregon (WSP 37455) but the spores that I saw in this material were narrower (**24**). I also found *D. tuberosa* on a herbarium sheet of *C. echinatus* collected ten miles west of Eugene, Oregon, collected by Helen M. Gilkey (WSP 46330 ex WS 221208). I found only one spore in this collection, measuring 86 × 10 μ. Recently I found the fungus common on this annual grass on Skinner's Butte, Eugene, Oregon (WSP 46573) and on Blanton Heights, Eugene (WSP 46575).

There is a fragment of *D. tuberosa* on *Cynosurus elegans* Desf. from Granada, Spain. It was collected in June, 1900 by E. Reverchon and

distributed by A. Kneucker in Gramineae exs. V. Lufurung 1901 n. 134. It was removed from WS 114290 and filed as WSP 46332. The fragment consists of a few very dark brown lesions bearing stout brown conidiophores 100–140 × 10–14 μ. I saw one plasmolyzed spore 80 × 9.5 μ and a few immature ones.

BIPOLARIS sp. occurs on a fragment of a dead leaf of *Cathestecum erectum* Vasey and Hack. collected by E. Palmer at Alamos, Sonora, Mexico, September 1890 (WSP 46333 ex WS 3030). A few straight or slightly bent, brown cylindrical but tapered spores 56–71 × 9.5–12.5 μ are borne on somewhat geniculate scattered conidiophores about 50 × 7–8 μ. The spores were multiseptate, one having 11 cross walls. This fungus did not have the yellow subhyaline spores of *D. tuberosa* being nearer to the color of *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoemaker (*Helminthosporium sativum* Pamm., King & Bakke). Since the host belongs in a group of grasses which includes *Bouteloua* and *Buchloe*, I compared the Mexican fungus with *B. buchloes* (Lefebvre & A. G. Johnson) Shoemaker (**9**, **16**). While the spore sizes are comparable, our fungus seems to have somewhat straighter, stiffer appearing spores than *B. buchloes*. They also lack the rounded apex. Not long after studying this fragment, I talked with Dr. Shoemaker in Montreal and found that he was interested in the group. He placed the Mexican specimen in *B. buchloes* but noted some differences (letter).

Associated with the *Bipolaris* on *Cathestecum*, above mentioned, were a few spores of CURVULARIA LUNATA (Wakker) Boed., probably saprophytic.

BIPOLARIS CYNODONTIS (Marig.) Shoemaker occurs on some dead leaves of *Cynodon dactylon* collected in Edwards County, Texas (WSP 46346 ex WS 168484).

DRECHSLERA BROMI (Died.) Shoemaker occurs sparsely on leaves of *Bromus laevipes* Shear collected by Sherl M. Dietz in the Plant Introduction Nursery, Pullman, Washington in 1958 (WSP 46358 ex 1956. Nursery No. 232-219, original seed source Marin County, California). This brome is an unreported host for *D. bromi*.

DRECHSLERA STENACRA (Drechsl.) Shoemaker is the cause of a mold on dead basal leaves of actively growing plants of *Agrostis alba* L. collected along woodland roads near French Creek, Snohomish County, Washington (WSP 46386). The nearly hyaline spores were mostly about 115 × 15–16 μ, 3–4-septate. Rather scanty material of *D. stenacra* was found on *A. tenuis* at Comax Lake, Vancouver Island, B. C., August 21, 1960 (WSP 46604).

The *Bipolaris*-like stage of PODOSPORIELLA VERTICILLATA O'Gara

occurs on leaves of *Danthonia pilosa* R. Br. collected on Bald Mtn., Humboldt County, California by Joseph P. Tracy (17267) on June 28, 1942 (WSP 46414 ex WS 160579). It also occurred on *D. spicata* (L.) Beauv. at Bruce Mines, Ontario (WSP 46504) and on *D. californica* Boland, Sweetgrass County, Montana, collected by C. L. Hitchcock (16544) on July 17, 1947 (WSP 46556 ex WS 175372). Drechsler described this fungus as *Helminthosporium cyclops* (**2**). He also illustrated the stick-like conidial structures which place it in *Podosporiella*. In addition, Brittlebank and Adam found a similar conidial form in Australia (**1**) with a stalked perithecium described by them as *Pleosphaeria semeniperda*. The *Podosporiella* phase occurs on seeds of grasses, cereals and other plants in the Palouse region of Washington. Among other collections is one on *Bromus tectorum* L. from Ravalli County, Montana, collected and determined by W. B. Cooke (WSP 41928). This whole group of collections probably warrants further study particularly because most of the filed material on *Danthonia* is a facsimile of *Bipolaris*. Shoemaker does not mention *P. verticillata* in his excluded species (**16**). The inexperienced would be hard pressed to identify the *Bipolaris* phase on moldy *Danthonia* leaves unless he was familiar with the literature cited above (cf also **18**, p. 380–381).

OVULARIA PUSILLA (Ung.) Sacc. & D. Sacc. occurred commonly on *Festuca rubra* L. near Notre Dame de la Merci, Quebec Prov., Canada (WSP 46469). Conidial tufts occurred in lines on discolored necrotic leaves. The same fungus was abundant on *F. elatior* L. causing numerous small elliptical eyespots on the leaves at Fleetwood, Minnesota (WSP 46619).

The aquatic grass, *Catabrosa aquatica* (L.) Beauv., is frequently attacked by *Puccinia poae-sudeticae* (Westd.) Jorstd. (**28**). I have a specimen found on a phanerogamic sheet collected by Hitchcock and Muhlick twenty miles east of Townsend, Montana, which shows a heavy attack of *Darluca filum* (Biv.) Cast. on the rust (WSP 46337 ex WS 159109). The combination of the rust with its parasite resulted in a leafspot effect that is confusing in the field.

What appears to be immature material of PHYLLACHORA GRAMINIS (Pers.) Fckl. occurred on *Cinna arundinacea* L. at Newcastle, Indiana, collected by Ray C. Friesner, September 28, 1935 (WSP 46351 ex WS 73185). Of more interest is the presence of an abundance of pycnidiospores in the developing stroma. These spores are faintly yellow or yellow-green, clavulate-filiform, pointed at the apex, blunter but tapered at the base, somewhat sinuous, 40–68 × 3–5 μ, 4–9-septate. Orton (**14**) did not mention any conidial stage in discussing *P. graminis* nor do

Tehon and Daniels in describing *P. cinnae* (**32**). Orton recognized the problem of the identity and relationship of the associated conidial fungi but usually avoided specific references to them in relation to the ascocarps. It is indicated in Friesner's material that the pycnidial fungus was probably part of the life cycle of the tar spot fungus. This cannot be proven, as yet. The *Septoria*-like fungus is not in the *Septoria avenae-Stagonospora arenaria* group. Except for their virtually hyaline color the spores are closest to those of *Phaeoseptoria festucae* var. *muhlenbergiae* (**20**). Saccardo (**15**, v. 2, p. 602) lists terete-falcate spermatia 16 × 1.5–2.0 μ which is the common kind of conidia usually associated with tar spots.

EPICHLOE TYPHINA (Pers. ex Fr.) Tul. was found on *Agropyron subsecundum* (Lk.) Hitchc. in the Plant Industry Nursery, Pullman, Washington by Sherl M. Dietz in 1958 (WSP 46359 ex 1956 Nursery No. 232-144, original seed source, Utah).

CLAVICEPS PURPUREA (Fr.) Tul. was found on *Elymus innovatus* Beal in the Plant Industry Nursery, Pullman, Washington by Dietz in 1958 (WSP 46360 ex 1956 Nursery No. 232-282, original seed source, Montana). A trace of *C. purpurea* was seen in the field near Sowerby, Ontario on *Glyceria canadensis* and it was present on *Elymus mollis* at Kelsey Bay, B. C. (WSP 46607).

ERYSIPHE GRAMINIS DC ex Merát was common on *Agrostis exarata* Trin. at Vancouver, B. C. (WSP 46605).

MYCOSPHAERELLA CINNAFOLIA Sprague occured on *Cinna latifolia* (Trevir.) Griseb. near Munger, Minnesota, August 11, 1959 (WSP 46625). The light brown perithecia with dark ostiolar tissue were slightly larger than those of the type, ranging up to 120 μ in diameter. Most of the spores were immature, but a small percentage of them were septate and as much as 15 × 3 μ in size. Except for the type from Oregon, this is the only collection seen of this fungus. The numerous perithecia occur on gray-brown dead leaves and are obscure.

PUCCINIA GLUMARUM (Schm.) Eriks. and Henn. was noted on *Phleum pratense* at Riffe, Washington (WSP 46621).

PHYSODERMA GRAMINIS (Büsgen) Fisher caused a dwarfing and brown stripe disease on *Agropyron repens* (L.) Beauv. at the Experiment Station, Vancouver, Washington. Specimens were obtained on November 4, 1959 (WSP 46484). Sporangia measured 20–38 × 21–35 μ which is virtually identical to the measurement for sporangia of *P. graminis*. G. W. Bruehl informed me that he has seen this fungus on quack grass at Pullman, Washington, but no specimen has been seen by the writer except the Vancouver material.

RHIZOCTONIA SOLANI Kuehn is common on decayed leaves and roots of cereals and grasses in western Washington (**17**). It sometimes causes a serious stem canker known as sharp eyespot (**20**, Fig. 7). This condition was common on a number of grasses along woodland roads near French Creek, Snohomish County, Washington, June 11, 1959, following and during prolonged rains. It was collected on: *Elymus glaucus* Buckl. (WSP 46382); *Dactylis glomerata; Phleum pratense; Poa pratensis; P. compressa* L.; *Festuca rubra* L.; *Bromus carinatus*; and *B. vulgaris*. None of these hosts are new to *R. solani* but the sharp eyespot strain of the fungus which I have called race 5 (**17**) has been noted previously only on *Dactylis glomerata* from the area. Later, on November 5, 1959, basal eyespots were noted on *Phalaris arundinacea* at Ajlune, Washington (WSP 46487). Sharp eyespot was also seen on *Poa compressa* near Campbell River, B. C. (WSP 46614).

TYPHULA INCARNATA Fr. caused stunting and death of fall-emerged annuals and leaf death on perennial grasses in small clearings along French Creek, Snohomish County, Washington. Evidence of leaf scald of basal leaves and a crown decay were still visible on June 11, 1959. Scattered to common small reddish sclerotia were present. Newly reported hosts for the state included the following: *Aira caryophyllea* (WSP 47370); *Festuca dertonensis; F. subulata; Holcus lanatus* L.; *Deschampsia elongata* (Hook.) Munro (WSP 46381, 46388); *Poa annua* (WSP 46391) and *P. compressa*. The fungus was also relatively common on *Agrostis alba* L., *A. palustris* Huds., *Poa pratensis, Festuca rubra* and incidental on *Elymus glaucus*. There had been very little snow during the winter, which locally is usually mild and wet. *Fusarium nivale* occurred with the *Typhula*, especially on dead plants of *Poa annua* and *Festuca dertonensis*.

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EFFECT OF ENVIRONMENT ON GERMINATION OF ASCOSPORES OF URNULA CRATERIUM [1]

CHARLES L. FERGUS AND RICHARD D. SCHEIN [2]

(WITH 6 FIGURES)

Urnula craterium (Schw.) Fr. is believed to be the perfect stage of *Conoplea globosa* (Schw.) Hughes (**6**), (*Strumella coryneoidea* Sacc. & Wint.), which causes an important canker disease of oak and several other hardwoods (**5**). Germination of ascospores of *U. craterium* has not been studied critically, although Davidson (**5**) observed that 90 to 100% of the ascospores shot from an apothecium germinated within 3 to 4 hours on cornmeal agar. Ascocarps from felled bur oak, *Quercus macrocarpa* Michx., in the spring of 1959 provided fresh material for investigation of ascospore germination. The possible importance of ascospores in long distance spread of the canker disease fungus stimulated this study.

MATERIALS AND METHODS

Segments of the ascocarps of *U. craterium* were attached to the lid of a petri dish. Expulsion of mature ascospores was obtained on the medium. Sterile spore prints on black paper were obtained similarly, the spores were pinkish buff in mass.

The influence of temperature on germination and growth was studied at various temperatures in Thelco Precision incubators. Each temperature was accurate to ± 0.5 degrees. Per cent germination was determined by observing 300 spores in at least three separate randomly chosen low power fields (100 ×) of the microscope. The per cent germination and the lengths of the germ tubes are in TABLE I. Growth was determined by measuring the diameter of the colony produced in one week. The media used were malt extract agar (17 g Difco malt extract, 17 g agar in one liter H_2O), Barnett's medium (**1**), and 17% water agar.

[1] Contribution No. 265 from the Department of Botany and Plant Pathology, Pennsylvania Agricultural Experiment Station. Authorized for publication July 25, 1960, as paper No. 2475 in the Journal Series.

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The pH of the malt extract agar (pH 5.3) was adjusted to 2.5, 6.5, and 9.5 with NaOH or HCl. A Beckman pH meter was used to determine pH values.

The influence of quality of light on germination was investigated by using lights patterned after those designed by Withrow and Elstad (**10**). Lamps were mounted over a hole $5\frac{3}{4}$ inch square in a light-tight chamber. Corning light screens 6 inches square gave the wave length desired. The screens used were Violet No. 511, Sextant Green, Signal Red, and Clear Noviol 0. An electronic photometer was used to determine energy levels. A graph depicting the relative sensitivity of the photometer across the visible spectrum was used to determine the number of layers

TABLE I

EFFECT OF TEMPERATURE AND MEDIUM[a] ON GERMINATION AND MAXIMUM LENGTH OF GERM TUBES[b] OF ASCOSPORES OF URNULA CRATERIUM

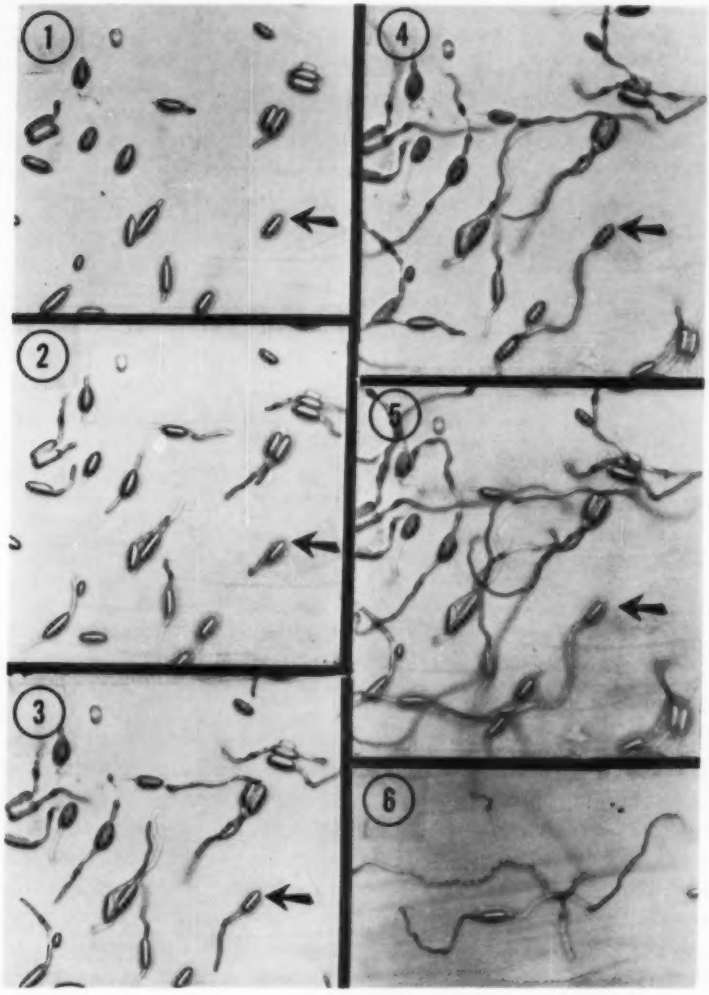
Temperature °C	Malt extract		Barnett		Water agar	
	Per cent	Germ tube length (μ)	Per cent	Germ tube length (μ)	Per cent	Germ tube length (μ)
5	98	55	35	44	—	—
10	60	220	30	180	—	—
15	92	600	50	300	25	100
20	100	1000	70	1000	73	550
24	92	1100	66	800	79	440
27	86	1200	82	1000	75	660
30	96	1400	100	800	85	220
35	2	66	51	12	0	0

[a] Observations were made 16 hours after ascospore discharge.
[b] The length given is the longest germ tube observed.

of 24 × 20 mesh cheesecloth to be used as neutral density filters for each compartment for approximate equalization of the energy levels. Baffled air filters in the front and back of each chamber ensured air circulation. The temperature of the room was 25 ± 1° C. The temperature in the chambers was monitored with thermocouples and a potentiometer and it remained constant. The response to light was determined by exposure to white fluorescent lights yielding 40 ft-c at plate levels. A light-tight box at the same temperature provided the control.

The longevity of ascospores exposed to white fluorescent lights of 40 ft-c at plate levels and to darkness was determined by exposure of spore prints to these conditions at 20–25° C. Spore prints were tested at various time intervals by streaking the spores onto malt extract agar.

The relation of time to germination was determined on malt extract



FIGS. 1–6. *Urnula craterium*. FIG. 1. Ascospores 2 hours after discharge onto hardened malt extract agar and incubated at 28° C. FIG. 2. Photomicrograph of the same area 3½ hours after discharge. FIG. 3. After 4½ hours. FIG. 4. After 5½ hours. FIG. 5. After 7 hours. The arrows point to the different stages of germination of the same spore. FIG. 6. Hyphal development of a single ascospore 22 hours after discharge. Note that a germ tube has been produced from each end of the spore, but one has become much more extensive and branched than the other. All × 167.

agar in the light at 28° C. Photomicrographs[3] (FIGS. 1–6) were obtained at intervals in the germination process.

EXPERIMENTAL RESULTS

The response to temperature of ascospores of *U. craterium* was modified by the medium upon which germination occurred (TABLE I). The ability of some spores to germinate at 35° is interesting since extrusion of protoplasm followed soon after exposure.

TABLE II

GROWTH[a] OF URNULA CRATERIUM AFTER 7 DAYS AT DIFFERENT TEMPERATURES AND NUTRIENT CONDITIONS

Temperature °C	Malt extract	Barnett[c]	Water agar
5	None visible	None visible	None visible
10	Barely visible[b]	None visible	None visible
15	40 × 50	30 × 40	None visible
20	45 × 45	60 × 65	None visible
24	45 × 55	85 × 85	Barely visible[b]
27	60 × 65	85 × 90	None visible
30	Barely visible[b]	Barely visible[b]	None visible
35	None visible	None visible	None visible

[a] The diameters are the averages of three cultures each and include the spore deposit area.
[b] Limited to the spore deposit area.
[c] The mycelial mass was thinner on Barnett's than on malt extract agar.

The ascospores of *U. craterium* germinated without external nutrients, although the percentage of spores germinating was reduced. Germ tube elongation was not as rapid without nutrients and no visible growth resulted.

The germ tubes were of less diameter on water agar than on the other two media; they were also more crooked, and more difficult to measure. At 35° C on all three media the cytoplasm was extruded, leaving spores and germ tubes empty. Most of the spores produced one germ tube, but about 20% produced two, one at each end of the spore (FIG. 3). The spores swell slightly just as the germ tube forms. When freshly expelled, the ascospores averaged 13 × 32 μ and at germ tube emergence 16 × 44 μ.

Allowing ascospores to shoot down onto agar from a small piece of the apothecium resulted in discharge onto an area averaging 10 mm sq.

[3] Appreciation is hereby expressed to Dr. H. B. Couch for his aid in this phase of the investigation.

Radial growth from this area was measured seven days after ascospore discharge on the three nutrient conditions and at eight temperatures, see TABLE II. Radial growth was faster on Barnett's medium, but the colony was thinner than on malt extract agar. Temperatures that allowed good germination (5, 10, 30 and 35° C) did not allow good growth.

Germination occurred over a wide pH range on malt extract agar, see TABLE III. Neither quality nor quantity of light affected germination which was 97 to 100% under all conditions.

The longevity of ascospores stored on sterile paper in petri dishes at 20–25° C in the light and in darkness was quite remarkable. After 383 days, germination on malt extract agar was 10% for ascospores stored in the light, and 40% for ascospores stored in the dark.

TABLE III

THE EFFECT OF pH ON GERMINATION[a] OF ASCOSPORES OF URNULA CRATERIUM

pH	Per cent germination	Maximum germ tube length in microns
2.5	30	400
5.3	100	1000
6.5	86	880
9.5	28	200

[a] Observations were made on malt extract agar 16 hours after ascospore discharge and incubation at 20° C in the dark.

The rapidity of germination of ascospores of *U. crateriu*m is remarkable (FIGS. 1–6); some spores germinated in 1½ hours. The germ tube of one spore in FIG. 1 measured 40 μ after 2 hours.

DISCUSSION

*Urnula crateriu*m is an operculate discomycete. Compared to *Sarcoscypha coccinea* (Scop. ex Fr.) Lambotte, another species in the same series, ascospore germination was relatively rapid, requiring only 1½ hours compared to 48 hours for *S. coccinea* (**8**).

The rapidity and high percentage of germination of ascospores of *U. crateriu*m are in marked contrast to the behavior of the conidia of its supposed imperfect state, *Conoplea globosa* (*Strumella coryneoidea*). The conidia of the latter have never been germinated (**2**, **9**). The ascospores therefore assume great importance in dissemination, for they are produced in abundance, have high viability, and tolerate a great range in pH.

A relatively large percentage of ascospores survived more than one year exposure to light and darkness at room temperature as compared

to a maximum longevity of seven months for ascospores of *Pyrenophora bromi* (Died.) Drechs. (3).

Light has been reported to inhibit spore germination, to increase germination, or to be necessary for germination to occur (4). There are many more reports, however, that light has no influence on spore germination (7). The ascospores of *U. craterium* did not require light to germinate, nor were there any observed differences in germination under different qualities of light.

SUMMARY

Ascospores of *Urnula craterium* were subjected to various environments to determine their effect on germination. Percentage germination and length of germ tube were used to determine the response. Ascospores germinated over a wide temperature range, including 5° and 35° C. The optimum, based on germ tube elongation and percentage germination 16 hours after expulsion from the apothecium, was 20–30° C. Germination of some spores occurred at 35°, but the protoplasm was extruded from both germ tubes and ungerminated spores. There was no subsequent growth. Added nutrients increased germination, but 85% of the ascospores germinated without them. There was no subsequent growth, however. Growth from germinated ascospores was excellent on malt extract agar and Barnett's agar; the optimum for continued growth was 24–27° C. Ascospores germinated over a range of pH from 2.5 to 9.5; the optimum was 5.3 for germination and germ tube elongation. Light had no effect on germination, but it affected longevity. Germination of ascospores stored 383 days at 20–25° C was 10% for storage in light and 40% for storage in dark. The ascospores germinate in less than 2 hours and germ tube elongation is rapid at optimum temperatures.

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STUDIES ON THE ISOLATION AND GROWTH OF PLANT RUSTS IN HOST TISSUE CULTURES AND UPON SYNTHETIC MEDIA. II. UROMYCES ARI-TRIPHYLLI [1]

VICTOR M. CUTTER, JR.

(WITH 3 FIGURES)

Previously (**2**), the isolation and axenic culture of 7 strains of the heteroecious rust *Gymnosporangium juniperi-virginianae* Schw. by the use of host tissue culture were reported. To include a monocotyledonous rust the technique was extended to the autoecious *Uromyces ari-triphylli* (Schw.) Seeler [2] parasitizing *Arisaema triphyllum* (L.) Schott. This combination of rust and host was selected because Morel and Wetmore (**4**) showed that some members of the Araceae might be capable of undifferentiated growth in agar culture. *U. ari-triphylli,* which is common on *Arisaema* and *Peltandra,* causes a systemic infection with the mycelium ramifying throughout the host and presumably overwintering in the corms and winter buds. Dufrenoy (**3**) and Rice (**7**) have discussed the structure of its haustoria and the development of the systemic mycelium. The behavior of the mycelium during growth of the host and the development of pycnia and aecia have been traced by Pady (**5, 6**). In 1952, I (**1**) presented a preliminary report on the growth of this rust in tissue cultures of *Arisaema triphyllum* and on its possible isolation in axenic culture upon synthetic media. Infection with mycelium of the isolated fungus had been accomplished at that time but it was not certain that the plants and tissue cultures were not already infected with *U. ari-triphylli.* Several of the controls subsequently did develop rust infections.

[1] The portions of the work reported here which were carried out from 1949 to 1952 were aided by a Rockefeller Foundation grant to the department of Plant Science of Yale University. The investigation subsequent to 1952 has been supported by a grant-in-aid from the National Science Foundation (NSF-G219). The very able assistance of Mrs. Rachael Harelson Roeder in the preparation of this manuscript is most gratefully acknowledged.

[2] Synonym: *Uromyces caladii* Farl.

TABLE I

COLLECTION DATA, AND HISTORY OF TISSUE CULTURES FROM CORMS OF ARISAEMA TRIPHYLLUM SYSTEMICALLY INFECTED WITH UROMYCES ARI-TRIPHYLLI

Locality	Date collected	No. of corms	No. of cultures	Contaminated 60 days	No. forming callus	No. dead 90 days	No. subcultured	Rust sporulating in culture	Uromyces isolated
	1949								
Bethany, Conn.	4/27	14	38	16	18	4	19	5	0
Branford, Conn.	5/1	30	83	62	14	7	0	6	0
Woodbridge, Conn.	5/6	68	107	46	52	9	88	21	1
Sharon, Conn.	5/10	32	79	30	47	2	34	13	0
Easton, Mass.	5/16	12	29	7	22	0	12	1	0
Keene, N. H.	5/30	17	37	18	16	3	12	2	0
	1950								
Bethany, Conn.	4/30	73	138	51	69	18	37	19	0
Lakeville, Conn.	5/21	64	91	32	46	13	0	2	0
Bethany, Conn.	6/27	16	49	12	14	23	6	0	0
	1951								
Woodbridge, Conn.	5/7	27	68	10	53	5	79	13	0
Farmington, Conn.	6/9	13	21	6	15	0	0	1	0
New Haven, Conn.	7/3	18	37	12	22	3	8	0	1
	1952								
Bethany, Conn.	4/26	30	68	21	36	11	45	2	0
New London, N. H.	6/15	10	27	4	22	1	30	1	—
	1953								
Greensboro, N. C.	4/1	39	76	14	49	13	21	0	0
High Point, N. C.	5/6	112	147	29	103	15	108	29	1
Greensboro, N. C.	6/21	14	26	4	21	1	6	1	0
Greensboro, N. C.	7/19	22	51	27	16	8	9	0	0
	1954								
Asheboro, N. C.	5/1	33	70	18	46	6	52	4	1
Fancy Gap, Va.	6/19	84	134	46	71	17	20	11	0
West Jefferson, N. C.	6/19	27	42	10	27	5	14	3	0
Greensboro, N. C.	7/20	16	21	19	2	0	5	0	0
Greensboro, N. C.	8/9	12	12	9	3	0	2	0	0
Greensboro, N. C.	10/12	16	18	14	2	2	0	0	0
	1955								
Greensboro, N. C.	5/4	48	81	19	57	5	29	2	1
High Point, N. C.	5/23	19	44	17	25	2	34	1	0
Greensboro, N. C.	6/20	26	47	24	20	3	26	0	0
Greensboro, N. C.	7/12	10	29	18	7	4	12	0	0
Total		902	1670	595	895	180	708	137	5

MATERIALS AND METHODS

Preliminary attempts were made to obtain systemically infected callus cultures from corms, roots, scapes, leaves, spathes, spadices, and fruits of infected plants of *A. triphyllum* and *Peltandra virginica* (L.) Kunth. The induction of monocotyledonous callus has been generally unsuccessful (**4**) but callus formation could be induced with ease in the tissues of the basal corm of *Arisaema*. No other tissues produced callus.

Experiments were carried out with corms of *Arisaema* from New England and North Carolina (TABLE I). The corms collected in spring and early summer were from plants which showed rust sori. Collections in the late summer and fall were from plants that had withered, but were from colonies which had been heavily infected with the rust. Some of the infected plants had been marked in June and then allowed to wither before the corms were collected in October.

The corms were washed, necrotic tissues were peeled away, they were soaked in full strength Clorox for 20 min., and then sliced. Sterile blocks of tissue 1 cm square and weighing 500–850 mg were placed upon Gautheret's agar with 10 μg/liter IAA or NAA (**2**). The cultures were kept at 20° C under 350 ft-c warm white fluorescent illumination until callus formed. These cubes of parenchymatous tissue were white at first (FIG. 1, A) but within 10 days became bright green and began to proliferate. The majority developed a cork cambium close to the cut surface (FIG. 1, B) and the desiccated cells at the surface sloughed off (FIG. 1, C). Thereafter, a brown periderm developed rapidly and soon became thick and relatively impervious (FIG. 1, D). These cultures ordinarily doubled their weight within 60–90 days but the increase ceased after 90 days as they became suberized and dormant. These dormant cultures remained viable and the internal parenchymatous tissues retained their ability to proliferate. Several cultures left in sealed tubes for 18–24 months, and then sectioned, were still green and internally viable. When aging cultures had developed a suberized periderm, this was stripped off aseptically and the internal parenchymatous mass was planted on fresh media (FIG. 1, E). Again, the cultures increased in size and weight for approximately 90 days until a new suberized periderm developed and they became dormant. By periodic stripping of the periderm and transfer to Gautheret's agar some cultures were maintained for better than 4 years.

It was difficult to demonstrate mycelium by sectioning and staining corms of *Arisaema* before starting cultures. Rice (**7**) commented on the apparent absence of mycelium in corms but Pady (**6**) reported abundant mycelium. The corm cells are packed with starch and raphides

which, along with the dense texture of the tissues, either limits or obscures the mycelium. After unsuccessful attempts to find mycelium in paraffin sections of infected corms, cubes of tissue were taken from the interior of the corms, washed in water, and minced for 5 min. in a Waring Blendor. The mycelium was then easily seen under the phase contrast microscope in wet mounts of the homogenate. This technique also demonstrated the presence of *U. ari-triphylli* in callus grown *in vitro* (FIG. 1, H). It was not always necessary to resort to the microscope since aecia frequently developed on explants. These sori produced aeciospores which, in the only test made, gave 41% germination on water agar 16 days after the appearance of the aecia.

OBSERVATIONS

On the 1670 cultures from infected corms, aecia were frequently produced during the first month but in 1 case 3 explants 7 months old produced aecia and aeciospores within 5 days after the periderm was stripped and the explants placed on fresh media. Since the cultures had been stored at 10° C in the dark for several weeks prior to stripping and transplanting, the possibility of producing sori at will by manipulating the cultural conditions was investigated. A number of cultures were exposed to low temperatures and varied light intensity but no consistent sorus formation was obtained, although the rust was verified by microscopic examination of the calluses at the conclusion of each experiment. There were indications that stripping the periderm and transplanting the calluses to fresh media, followed by increasing the light intensity from 350 to 890 ft-c induced aecia on cultures 9 months old, but here only a portion of the calluses produced aecia. Uredia, telia, and pycnia were never observed.

In 1 series from corms collected in Bethany, Conn., aecia appeared on 19 of 138 cultures within 3 weeks of their initiation. The cultures which had fruited were maintained by stripping and transferring at 6 months intervals. Microscopic examination showed mycelium in August, 1952, and still present, in undiminished amounts, in the calluses subdivided in December, 1953. Four of 14 subcultures showed mycelium in July, 1954, but none could be found in the other subcultures. When the 4 were discarded in July, 1955, no mycelium was detected in any of the subcultures. It was difficult to locate mycelium in sections of corms or calluses but, in those where mycelium was found, it was located in the parenchyma and not in the periderm, except that mycelium was present in the areas adjacent to and below the sori. In the later cultures, which were homogenized, the calluses were divided into peri-

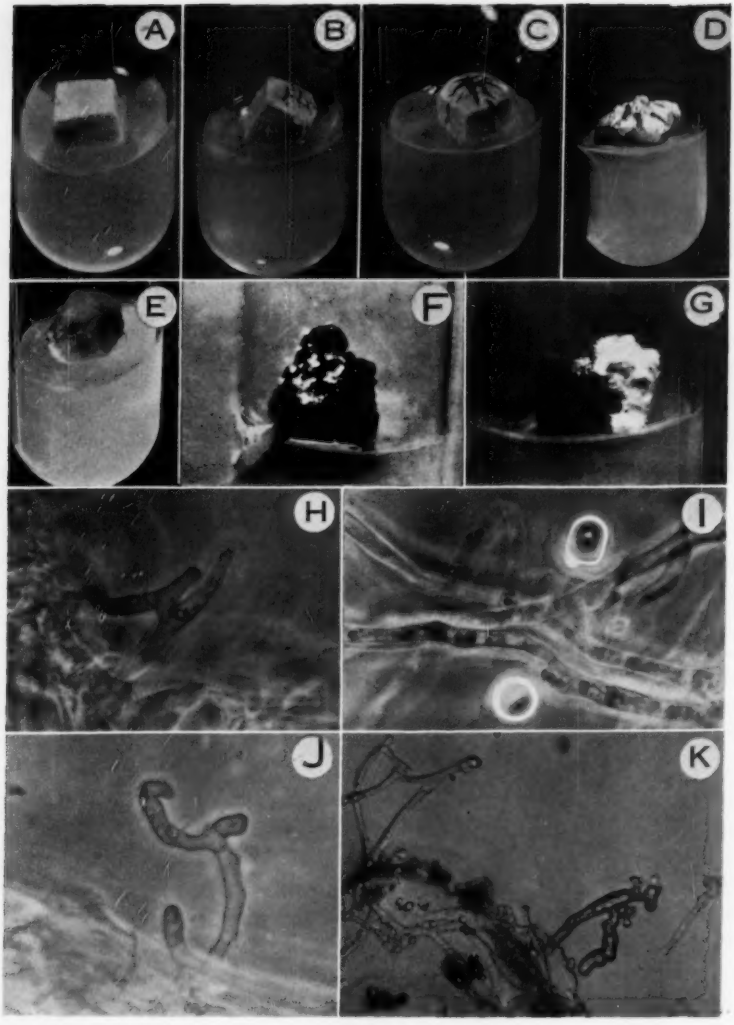

FIG. 1, A–K.

FIG. 1. Isolation of *Uromyces ari-triphylli* from systemically infected corms of *Arisaema in vitro*. A. Cube of corm tissue 5 days after initiation of culture. × 1.5 B. Culture 35 days old with dead surface tissue starting to slough. × 1.5. C. Culture 50 days old with periderm developing below dead tissues. × 1.5. D. Culture 90 days old with periderm well developed and dead tissues sloughed off. × 1. E. Primary subculture 120 days old with original periderm stripped at 90

dermal and parenchymatous portions. Mycelium was only found in the parenchyma. In other cultures the mycelium persisted in the parenchyma of proliferating explants up to 3 years even when the explants had become dormant. This demonstration of systemic mycelium in the corms and in subsequently formed callus probably invalidates Rice's suggestion (**8**) that this rust may repeat from soil-borne spores rather than from a systemic mycelium in the corms. Pady's observations (**6**) support my view.

The establishment of rust infected calluses uncontaminated with saprophytes was easier than with any other rust-host combination. Not only was the percentage of contamination in *Arisaema* corms lower, but the number of organisms was also limited. The most frequent contaminant was a species of *Xanthomonas,* tentatively identified as *X. conjaci* Uyeda, which was prevalent on corms collected in June and later in North Carolina. This bacterium ordinarily grew out of the host tissue cultures within 10 days from initiation and rapidly overgrew the cultures. *Erwinia aroideae* (Townsend) Holland was also isolated from the North Carolina collections (**9**). A species of *Alternaria* was the only fungus repeatedly isolated from the surface sterilized corms. Casual contaminants rarely appeared in the cultures after 60 days. Even during the removal of the periderm, few cultures became contaminated. If the outer tissues of the corm were carefully scraped away and the surface thoroughly wetted with Clorox, contaminants rarely appeared. The interior of the *Arisaema* corms was relatively sterile except for the mycelium of *Uromyces.*

Callus formation occurred most readily in corms collected during April, May, and June when contamination was at a minimum (FIG. 3). The average number of contaminations increased rapidly during the latter part of the growth period of *Arisaema* and was highest in dormant corms collected in the fall (FIG. 3). This is probably because an increasing proportion of the exterior tissue becomes necrotic as the corms approach dormancy. When growth is resumed in the spring these necrotic tissues are sloughed from the expanding corm and the major portion of the corm is comprised of healthy meristematic cells.

days. × 1.5. F. Mycelial pustules of *U. ari-triphylli* developing on corm callus 7 months old. × 4. G. Mycelium of *U. ari-triphylli* growing into medium from corm callus 8 months old. × 4. H. Mycelium from interior of callus culture shown in D. Dark phase contrast. × 830. I. Mycelium from group B callus 90 days after inoculation with C-307-49. The refractive structures are starch grains. Dark phase contrast. × 830. J. Mycelium from group A callus 122 days after inoculation with C-307-49. Dark phase contrast. × 830. K. Isolated mycelium of C-307-49 from axenic culture 10 days old on Gautheret's agar. × 520.

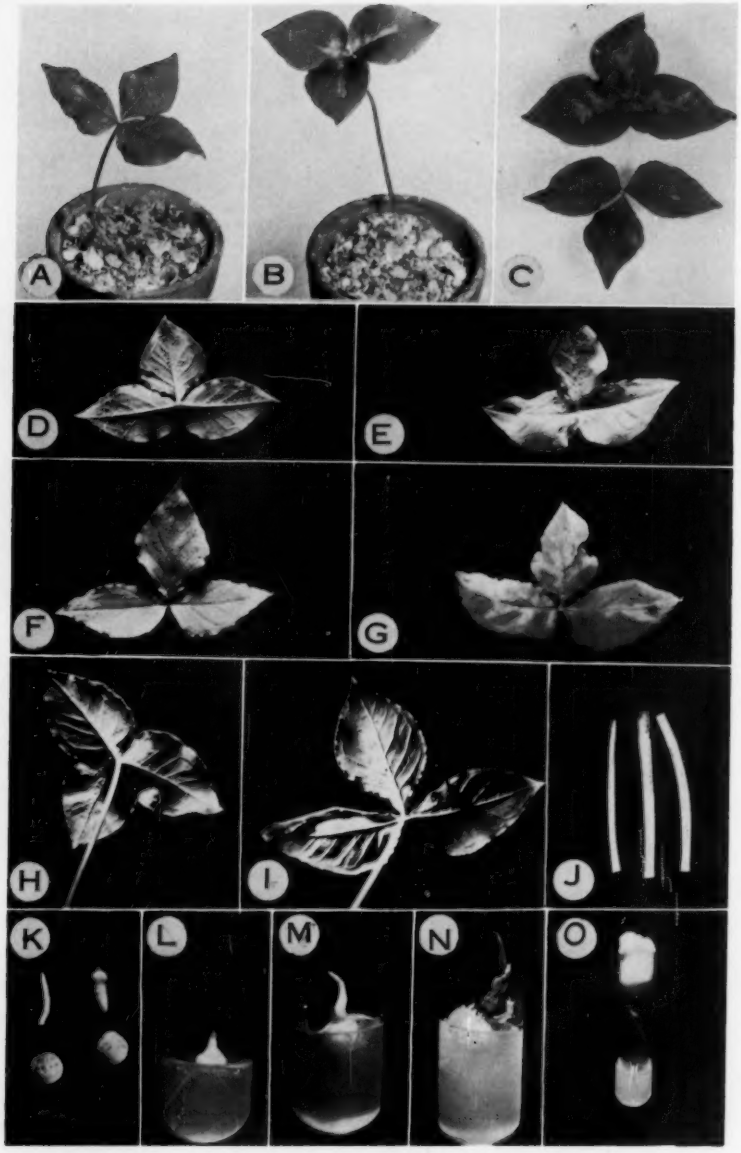

FIG. 2, A–O.

ISOLATION OF U. ARI-TRIPHYLLI

All cultures showing contaminants during the first 60 days of culture were discarded. The first rust-like organism to grow in the cultures appeared on Nov. 12, 1949, in a callus culture started from infected corms collected May 6, 1949, in Woodbridge, Conn. This culture developed aecia on June 1 and aeciospores until June 18. Subsequently the culture developed a thick periderm and by Aug. 1 the growth had slowed and the callus was stripped and transplanted. On Nov. 12, pale yellow mycelial pustules were evident on the callus which was still green but somewhat watery and translucent (FIG. 1, F). By Nov. 29, the pustules had coalesced and grown down to the agar (FIG. 1, G). Growth was slow and the mycelium did not cover the agar until Jan. 15, 1950. By then the callus was soft and watery but its cellular structure was preserved. Bits of mycelium from the periphery were transferred to Gautheret's media. As soon as the transferred organism, which was designated C-307-49, grew independently, the callus from which it had developed was dissected. The interior, throughout the periderm and partially into the parenchyma, was filled with intercellular mycelium. No haustoria could be distinguished in either fixed and stained or fresh material examined under the phase contrast microscope. In all other respects this mycelium resembled that found in corms known to be infected with *U. ari-triphylli* but it was present and proliferated more luxuriantly than in any *Arisaema* tissues previously encountered. While this mycelium was growing from the callus, and when the host tissues were macerated and examined, no spore-like structures were found. It was entirely vegetative and has remained so.

When this fungus was isolated in January, plants of *Arisaema* were

FIG. 2. Controlled inoculations on *Arisaema triphyllum* with axenic cultures of *Uromyces ari-triphylli*. A–B. Pycnia developing 36 days after inoculation with C-307-49 in greenhouse. C. Systemic aecial infection on plants inoculated in field with C-502-51. D. Aecia on plant inoculated in field with C-307-49. E. Pycnia on same leaf as D. F. Uredial pustules on leaf 77 days after greenhouse inoculation with C-307-49. G. Aecial infection on plant inoculated in field with NC-813-53. H. Aecia on plant in May, 1955, from colony inoculated in April, 1954, with C-502-51. I. Uredia and telia on plant inoculated in greenhouse with C-307-49; plant had previously borne aecia. J. Uredial pustules on petioles 77 days after inoculation in greenhouse with C-307-49. K. Uninfected young corms and winter buds of *Arisaema* scraped and prepared for culture. L. Sterile intact corm culture 24 days old with apical bud and roots developing. M. Scape sheath developing from sterile corm culture 48 days old. N. Plantlet with scape emerging, 61 days old from sterile corm. O. Sterile plantlet, 90 days old, with leaf unfurling.

not available and field testing was postponed, but a number of callus cultures from uninfected corms were on hand. Twenty-one uninfected calluses from 9 different corms were inoculated with the isolated mycelium as follows: The periderm was stripped to the cork cambium and the calluses were transferred to Gautheret's agar. Seven (Group A) were left otherwise intact and were inoculated by placing bits of mycelium on the agar surface where they grew. Seven more calluses (Group B) were cut in halves, exposing a section across both cork cambium and parenchyma and oriented with the cut section uppermost. These were inoculated with the mycelium, prepared by triturating a 10 day culture in Gautheret's solution (FIG. 1, K) in the Waring Blendor and concentrating the homogenate which was then spread over the cut surfaces of the calluses. The 7 remaining calluses (Group C) served as controls. All cultures were grown at 20° C under 350 ft-c illumination for 35 days after which 2 calluses from each group were sectioned and examined under phase contrast.

In Group A the mycelium had grown to and around the calluses but apparently had not penetrated the periderm. There was no evidence of internal mycelium or of appreciable mycelial penetration of the outer layers of the periderm. In the group B calluses there was luxuriant mycelium over the cut surfaces and it had penetrated deeply into the parenchyma. At 35 days these calluses showed the water soaked translucent appearance noted in the original culture. Group C calluses showed no internal mycelium or any sign of infection. No sori or spores were present on any of the groups.

The remaining calluses in each group were kept under the same conditions for an additional 60 days by which time the mycelium had reached the periphery of the tubes. Group A calluses were still firm, green to brownish on their peridermal surfaces, and showed no external sign of infection. They had not increased at a rate comparable to the controls, which had approximately doubled in volume, but they had developed a thick periderm. The group B calluses were invested with mycelium (FIG. 1, I) and they had disintegrated so that they could not be lifted unbroken from the tubes. The controls showed no infection.

The remaining 5 group A and 5 group C calluses were again stripped and transferred. Eight days after transfer 1 group A callus developed 4 aecia on the stripped surface and aeciospores were present 16 days later. Twenty-seven days after this transfer all the calluses in both groups were examined for the presence of mycelium within the tissues. None of the other calluses in either group had developed sori and mycelium was found only in the single group A callus which had sporulated.

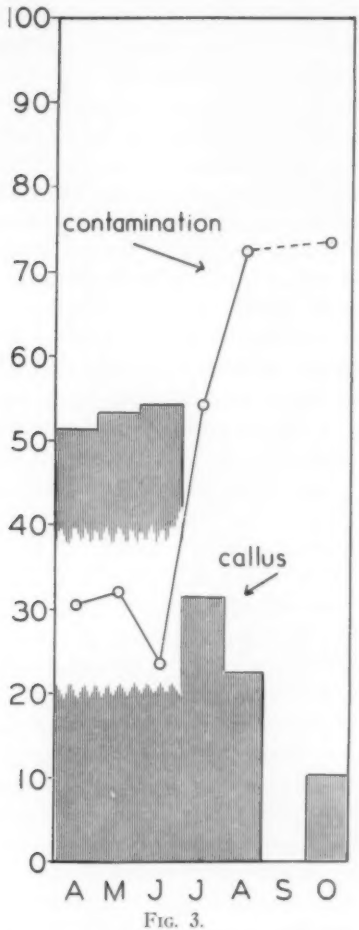

FIG. 3.

FIG. 3. Corms of *Arisaema triphyllum* systemically infected with *Uromyces ari-triphylli*. Average per cent contamination of corm callus cultures with saprophytic fungi and bacteria, compared with callus formation by months in which the infected corms were collected. Total of 902 corms from 28 localities represented. Collection and cultural data in TABLE I.

Here it was typical of the mycelium of *U. ari-triphylli* in systemically infected calluses (FIG. 1, J).

The appearance of aecia upon this single callus following inoculation with the isolated mycelium suggested that the isolated organism was

U. ari-triphylli and this was reinforced by the absence of aecia on the controls. None of the other group A calluses showed any sign of infection although they had been inoculated and grown in identical fashion. Also, as indicated earlier (1), there was no test by which a field-collected plant of *Arisaema* could be proved to be free of infection, since plants were known which showed no infection when aecia were on neighboring plants, but later developed aecia, uredia, or telia. The presumably uninfected calluses used for testing might actually have undetected systemic mycelium. There was also the question as to why the fungus inoculated on the group B calluses had behaved like a pathogen and destroyed the cellular structure of the tissues while the single infection in group A developed as a typical obligate parasite, sporulated and established a systemic infection with little detriment to the host cells.

The uncertainties connected with this single successful reinfection prompted extensive tests with the isolated fungus in April, 1950, as soon as *Arisaema* plants became available in the field. Colonies of plants were selected around New Haven, Conn., where no *U. ari-triphylli* had been observed in previous years. Plants in these colonies were carefully checked for aecial infection during April, 1950, and no plants were selected which were within 100 yards of an infected plant. Sixty corms from 7 uninfected colonies were collected and 110 callus cultures were started on April 23. Thirty days later all were green and had developed a periderm. Twenty-eight cultures were contaminated and were discarded. None of the remaining 82 cultures developed any sori of *U. ari-triphylli* during the first 30 days. At the end of this period the cultures were then transferred to Gautheret's media and 60 were inoculated with the putative isolate of *U. ari-triphylli* (C-307-49), the remaining 22 cultures serving as controls. The inoculum, consisting of a mycelial homogenate from a 15 day old culture of C-307-49 grown on oat digest agar (Ben Venue), was pipetted over the surfaces of the calluses. The cultures were sealed with parafilm and placed at 20° C under 350 ft-c illumination for 90 days. By then the fungus had virtually covered the calluses. None of the inoculated or control calluses developed sori during this time, nor were there spores on the external mycelium. The inoculated calluses averaged approximately half the size of the controls. All calluses were firm and there was no external sign of degeneration. At 90 days all the calluses were stripped aseptically and replanted but not reinoculated. Six of the inoculated and 1 of the control calluses were apparently dead. No mycelium was found in them. Of the remaining 54 inoculated calluses placed on fresh media, 23 developed mycelium, similar to the inoculum, on the surface of the

new media. This mycelium apparently originated from portions of the periderm which had not been removed. None of the control calluses were contaminated.

Nine days after stripping and transplanting, 4 of the inoculated calluses developed aecia and produced aeciospores. Three of these 4 sporulating cultures also developed mycelium on the surface of the agar. None of the controls developed aecia or mycelium. All 54 inoculated cultures and 21 controls were stripped and transferred at 3 month intervals for 1 year but none developed sori. By this time 29 of the inoculated calluses and 8 of the controls were dead. Both the healthy and dead calluses were individually triturated and examined for mycelium. One of the 4 cultures which had produced aecia showed internal mycelium indistinguishable from *U. ari-triphylli*. None of the others showed internal mycelium, although 7 of the inoculated calluses were accompanied externally by the fungus with which they were inoculated. This experiment supported the assumption that the isolated fungus was *U. ari-triphylli* but the low incidence of reinfection left many questions unanswered.

FIELD INOCULATIONS

In April, 1952, 12 uninfected colonies around New Haven, Conn., which had been under continuous observation during the previous year and were removed from any locus of *U. ari-triphylli* were selected. In 6 colonies, plants with the leaves just breaking through the scape sheaths and still furled were inoculated by inserting a few drops of an homogenized suspension of C-307-49 in Gautheret's solution into the open axil of the uppermost sheath with a sterile syringe. No attempt was made to inoculate subepidermally. Plants in the remaining uninfected colonies were inoculated by injecting the homogenate into the interior of the scapes just below the first leaf sheath with a sterile syringe. Inoculated plants were covered with pliofilm bags tied at the ground level. The inoculated plants and the other plants in the colonies which served as controls were observed throughout the summer. TABLE II presents the results of these inoculations.

The size and leaf expansion of the bagged plants was approximately normal. Development of pycnia, aecia, and aeciospores was normal and the distribution of sori on the leaves was characteristic (FIG. 2, D, E). In several cases where the inoculum was introduced into the scape, pycnia and aecia appeared on the petioles at their junction with the leaf sheath, but never below the point of inoculation, and none of the spathes or spadices developed sori. The rather scanty production of uredia and

telia on inoculated plants where aecia developed was not considered unusual since the summer was dry and the majority of the plants withered early in July. Uredia and telia were equally scarce on naturally infected plants.

The development of *U. ari-triphylli* on the inoculated plants left little doubt that the isolated C-307-49 was this organism but the appearance of sori on 5 of 90 uninoculated controls again raised the question of undetected systemic infection. But none of the controls had been bagged

TABLE II

FIELD INOCULATIONS ON UNINFECTED PLANTS OF ARISAEMA TRIPHYLLUM WITH MYCELIAL HOMOGENATE OF AXENIC CULTURE OF UROMYCES ARI-TRIPHYLLI C-307-49

Location of colony	Date	No. plants in colony	No. plants inoculated	Site of inoculation	No. producing aecia	Date	No. producing uredia or telia	Date	Uninoculated plants rusted	Date
New Haven, Conn.	4/17	12	6	Leaf sheaths	0	—	0	—	0	—
Bethany, Conn.	4/17	42	19	Leaf sheaths	7	5/20	1	6/30	2	7/3
Woodbridge, Conn.	4/18	7	3	Leaf sheaths	1	6/1	0	—	0	—
Oxford, Conn.	4/18	23	15	Leaf sheaths	0	—	0	—	1	6/24
New Haven, Conn.	4/22	8	4	Scapes	1	5/22	0	—	0	—
Bethany, Conn.	4/22	19	12	Scapes	3	5/30	1	7/9	0	—
New Haven, Conn.	4/23	6	4	Leaf sheaths	2	6/2	0	—	0	—
New Haven, Conn.	4/23	20	14	Leaf sheaths	2	6/1	0	—	1	7/4
Woodbridge, Conn.	4/26	9	6	Scapes	5	6/1	2	7/9	0	—
Bethany, Conn.	4/26	17	8	Leaf sheaths	1	6/12	0	—	0	—
Orange, Conn.	4/27	31	22	Leaf sheaths	9	6/2	3	7/9	0	—
Bethany, Conn.	4/28	29	20	Scapes	3	6/12	0	—	1	6/28
Totals		223	133		34		7		5	

and all of the sori on the unbagged plants developed later in the season than those on the inoculated plants. Had these been uredia or telia, they might have resulted from spores blown from earlier infections away from the immediate vicinity. All of the sori were aecia so that this explanation is unsatisfactory.

GREENHOUSE INOCULATIONS

In 1953, colonies of *Arisaema* were observed in the vicinity of Greensboro, and their development was followed until the plants withered. *Arisaema* is uncommon in the Piedmont of North Carolina and the incidence of *U. ari-triphylli* is low. In a number of the colonies no rust was detected and some were several miles from other colonies. After the plants in these isolated uninfected colonies had died they were

brought to the laboratory. The corms were washed, all necrotic tissues were removed and the corms were planted singly in pots. The pots were placed in a cool, dark cellar until February 1, 1954, when they were moved to a greenhouse maintained at approximately 20° C. On March 18, 42 plants were unfurling leaves and 30 were inoculated with C-307-49 by dropping the mycelial homogenate into the axil of the leaf sheaths. The remaining 12 plants were not inoculated and were separated as controls in a different part of the greenhouse. Both sets were then screened under pliofilm frames.

On April 26, 7 inoculated plants were producing pycnia, aecia, or both (FIG. 2, A, B) along the midribs and by May 2, 3 additional plants had developed aecia. No sori appeared on other inoculated plants or on the controls. On June 4, 3 of the 10 plants which had produced aeciospores also showed scattered uredia (FIG. 2, F, I, J) on the leaves and petioles. Some subsequently developed teliospores. The development of the plants in the greenhouse was poor and all were chlorotic. By July they had matured. The dormant corms were left in the pots and were returned to the cellar during the summer and fall. They were returned to the greenhouse in February, 1955. Only 5 of the 42 developed scapes and only 1 was from a corm which had borne a rusted plant the previous year. This infected plant produced a few pycnia and aecia on one leaflet on April 2, and shortly thereafter died. All plants were dead by May 7, 1955. Pady (**6**) had similar difficulty in maintaining *Arisaema* plants.

Attempts to grow plants from rust-free seeds placed on Gautheret's media were not successful. Fifty-six seeds were planted singly in tubes but only 4 germinated and none of the plantlets developed beyond 3 mm in height.

In many colonies, young plants developed each spring from small winter buds or immature corms. A number of these buds and small corms were collected in November, soil and adhering tissues were scraped off (FIG. 2, K), and they were immersed for 10 min. in Clorox. These were planted in tubes of Gautheret's media and within 30 days most of them produced miniature plants (FIG. 2, L, M, N). As far as could be determined the plants were sterile. Eighteen were inoculated on December 22 in the bud sheaths with a mycelial suspension of C-307-49 (FIG. 2, O). The mycelium proliferated rapidly and most of the plantlets soon died. But 3 of the plantlets continued to grow and, on February 8, 1 had a few aecia on the partially unfurled leaves. Leaf growth was not normal and the fungus developed only immature aecia. The other 2 surviving plants showed no infection and had withered by

March 1. Because of the inhibited growth of the plants in tubes the method was not used further but it offers promise.

FURTHER ISOLATIONS

Following the first isolation of *U. ari-triphylli* (C-307-49), a similar fungus (C-502-51) was isolated from corms collected in New Haven, Conn. Three other strains, indistinguishable morphologically, have been isolated from corms collected at High Point (NC-790-53), Asheboro (NC-813-53) and Greensboro, N. C. (NC-912-54). All of these appeared in callus culture after at least 80 days on Gautheret's medium. Their development in culture was identical with that of the original isolate, and collection data are in TABLE I.

TABLE III

FIELD INOCULATIONS ON UNINFECTED PLANTS OF ARISAEMA TRIPHYLLUM WITH AXENIC ISOLATES OF UROMYCES ARI-TRIPHYLLI. ALL PLANTS INOCULATED APRIL 24, 1954

Isolate used	No. of plants in colony	No. of plants inoculated	No. producing aecia	Date	No. producing uredia	Date	Uninoculated plants rusted
C-307-49	14	9	2	5/22	0	—	0
	19	14	1	5/29	0	—	0
C-502-51	6	3	0	—	0	—	0
	26	20	9	6/1	4	7/11	0
	16	11	3	5/22	0	—	0
NC-790-53	38	30	7	5/29	0	—	0
	13	9	1	5/22	0	—	0
NC-813-53	11	6	4	5/22	0	—	0
	5	3	2	5/29	0	—	0
NC-912-54	32	15	6	5/22	4	7/11	0
	12	6	1	5/29	1	7/11	0

These strains were tested by field inoculation in the spring of 1954 on uninfected colonies of *Arisaema* at Freeman's Mill, Guilford County, N. C. The inoculum was dropped into the axils of unfurling leaves. This station is isolated from other stands of *Arisaema* and, since no infected plants had been found there, the inoculated plants were not bagged, but separate colonies were used for testing each isolate. The results are summarized in TABLE III.

The colonies which had been inoculated were revisited in 1955 and

1956 while aecia were being produced, and later in June when uredia were present in other stations. In 1955, 1 colony infected with the isolate from Woodbridge, Conn. (C-307-49) and 2 colonies with the New Haven, Conn., isolate (C-502-51) showed respectively 1, 4, and 2 plants bearing aecia (FIG. 2, H). No other infected plants were found. In 1956, 1 colony previously inoculated with C-502-51 showed one small plant bearing pycnia and aecia (FIG. 2, C) but no other infections were present. In subsequent years, 3 infected plants have been found in this colony. These tests were not extensive enough to be conclusive but they suggest that it may be possible to initiate perennial infections with isolated strains of *U. ari-triphylli*. This seems probable since the controls in the 1954 inoculations never developed rust.

DISCUSSION

The series of successful reinfections of *Arisaema* with 5 isolated strains of *U. ari-triphylli* indicate that it is possible to obtain host infection with the homogenized mycelium of this organism after axenic culture in the absence of host cells. These isolates, to some degree, retain their ability to reinfect their host after prolonged saprophytic culture. Like the isolated strains of *Gymnosporangium* (**2**) they apparently pass successively from a condition of obligate parasitism to a transient destructive pathogenesis and then to a heterotropic saprophytism during the period of their emergence from the host tissue cultures. As in *Gymnosporangium*, it is obvious that very few of the cells of *U. ari-triphylli* undergo this transformation. Furthermore, the time at which this transformation will occur has not been predictable.

The 1670 systemically infected tissue cultures which were started and the number of rust cells and nuclei which were given the opportunity to utilize the noncompetitive, nutritionally adequate environment of the tissue culture medium, in comparison with the number of isolated strains which could grow in this environment lead to the conclusion that a very small percentage of cells or nuclei of *U. ari-triphylli* possesses the ability to grow saprophytically. If the assumption is made that each saprophytic strain arose from a single cell, or perhaps a single nucleus, then it is obvious that the mutation which allowed these cells to utilize the synthetic culture medium is rare. Since the number of rust cells per corm of *Arisaema* is variable and difficult to estimate, the data do not allow a valid analysis of the mutation rate. It can only be assumed that this mutation does not occur frequently or that it is not easily detected.

The similar behavior of *U. ari-triphylli* to *Gymnosporangium* suggests that the transformation to saprophytism may occur in other species of the Uredinales. If so, a more effective procedure might yield a higher frequency of saprophytic strains. The behavior of saprophytic strains after reinfection of the host, and the percentage of recovery after host passage, should determine whether they retain their saprophytic proclivities or revert to the obligate condition.

SUMMARY

Callus cultures from corms of *Arisaema triphyllum* systemically infected with *Uromyces ari-triphylli* were maintained for several years by subdivision and transfer. The rust has produced aecia upon these calluses. Five strains have been isolated and grown in axenic culture. These isolated strains have been maintained on synthetic media and all were capable of reinfecting *Arisaema* in tissue culture and under field conditions and of forming characteristic sori with viable spores on the inoculated hosts.

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NEW SPECIES OF ENTEROBRYUS FROM SOUTHEASTERN UNITED STATES[1]

ROBERT W. LICHTWARDT

(WITH 17 FIGURES)

Three new species of the obligate endocommensal genus *Enterobryus* were discovered some years ago living within the hindguts of millipeds collected in the southeastern part of the United States. Like other members of the Trichomycete order Eccrinales, these species grow attached to the chitinous gut lining of their hosts, and produce one or more spore types at the terminal end of the unbranched, coenocytic, filamentous thalli.

The descriptions which follow are based upon material prepared according to methods previously presented (Lichtwardt, **1954**). The plates were made from thalli mounted in lactophenol cotton blue.

Enterobryus dixidesmi Lichtwardt, sp. nov.

Thallis firme spiralibus per singulas longitudines, aut plerumque propinquis basi flexis; usque ad 1.5 mm long., saepius minus long.; 5–17 μ diam, 9–13 μ typico. Cytoplasmate hyalino et aliquantum granoso. Caulis retinaculi saepe brevissimus, cum vel sine orbi basali. Sporangiosporis (typo A) circa 40–90 × 9–11 μ. Sporis uninucleatis (typo F) 8–14 × 9–13 μ. Sporis ellipsoideis (typo I) 18–23 × 5–6 μ.

Thalli tightly coiled throughout their length, or curved predominantly near the base; length up to 1.5 mm, usually much shorter; diameter ranging from 5–17 μ, typically 9–13 μ. Cytoplasm hyaline and somewhat granular. Holdfast stalk usually very short, with or without a basal disk. Sporangiospores (type A) about 40–90 × 9–11 μ. Uninucleate sporangiospores (type F) 8–14 × 9–13 μ. Ellipsoid spores (type I) 18–23 × 5–6 μ.

Attached to the hindgut lining of the milliped *Dixidesmus tallulanus* Chamberlin. R. W. Lichtwardt slide H-4-6, TYPE, in author's collection.

[1] This paper is based upon a portion of a Ph.D. thesis submitted to the University of Illinois in 1954. I am indebted to Dr. Leland Shanor who supervised this research, and also thank Miss Thelma Howell, Executive Director, and the Highlands Biological Station for a grant from the Col. and Mrs. John S. Sewell Research Fund during the summer of 1952. I am grateful to Dr. Richard L. Hoffman for identifying specimens of the millipeds, and to Dr. Austin Lashbrook, Department of Latin and Greek, The University of Kansas, for his assistance in preparing the Latin diagnoses.

Dixidesmus tallulanus is a rather small, fragile milliped known only from northern Georgia (Rabun County), western North Carolina (Macon County), and the Great Smoky Mountains in Tennessee (Sevier County) (Chamberlin and Hoffman, **1958**). I collected about 19 individuals from a number of localities on the Highlands Plateau, Macon County, and 9 of these were infected with thalli of *Enterobryus dixidesmi.*

The general appearance of the hyphae of *E. dixidesmi* is very similar to *Enterobryus moniliformis* (Leidy) Lichtwardt from the milliped *Scytonotus granulatus* (Say) (Leidy, **1850**; Lichtwardt, **1957**), but the morphological differences are considered sufficient to warrant regarding the fungi as different species. In brief, the sizes of the thalli and reproductive structures of *E. dixidesmi* (FIGS. 1–8) are larger on the average than those of *E. moniliformis,* except for the length of the filaments, which may become about twice as long in the latter species. The most obvious difference between the two species is the structure of the holdfasts. Whereas the holdfasts of *E. dixidesmi* normally consist of a basal disk with no stalk (FIG. 3), those of *E. moniliformis* usually show little basal enlargement and have a relatively long, striated stalk with a constriction just below the attachment to the hypha (Lichtwardt, **1957**, Figs. 3, 4).

It is interesting to note that the two millipeds in question, bearing such similar gut fungi, belong to the same family, the Polydesmidae. Both species can be found in parts of North Carolina, but *Scytonotus granulatus* ranges north to New York and as far west as Iowa and Missouri (Chamberlin and Hoffman, **1958**).

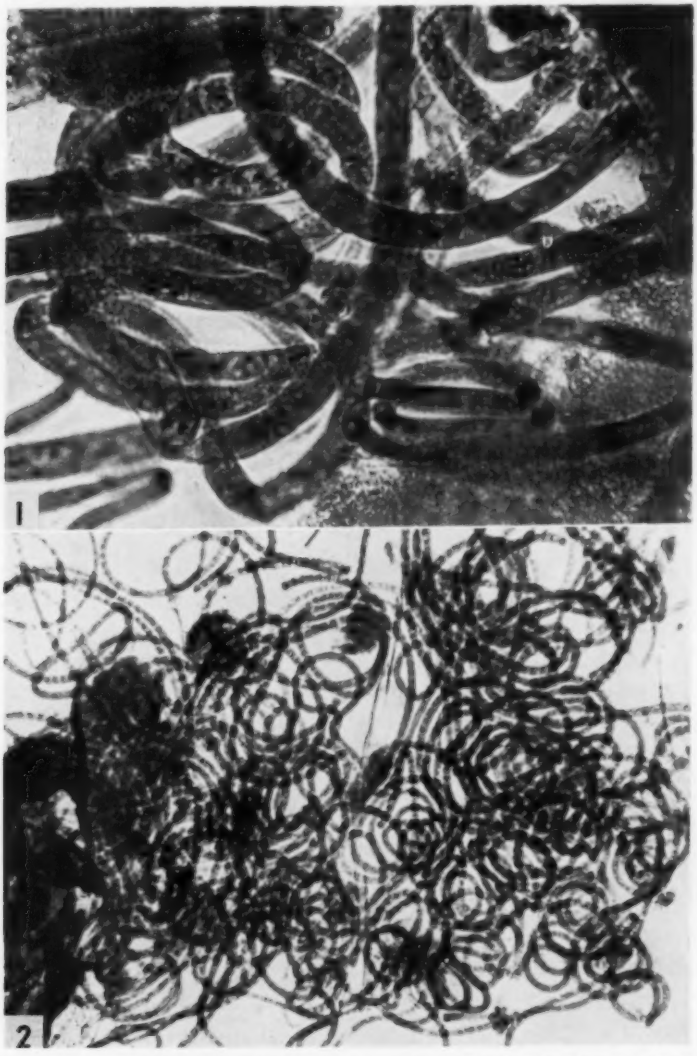
The coiled nature of the hyphae of *E. dixidesmi* (FIGS. 1, 2) seems to be found only in hosts with abundant infection. The thalli present in hosts with little to moderate infection show only slight spiraling, or merely are curved near the base.

Multinucleate sporangiospores of type A (FIG. 4) were abundant in the material examined. All stages of maturation and germination were seen. These spores escape laterally from the proximal end of the sporangium, and germinate after attachment to the gut lining of the same host in which they were produced (FIG. 7).

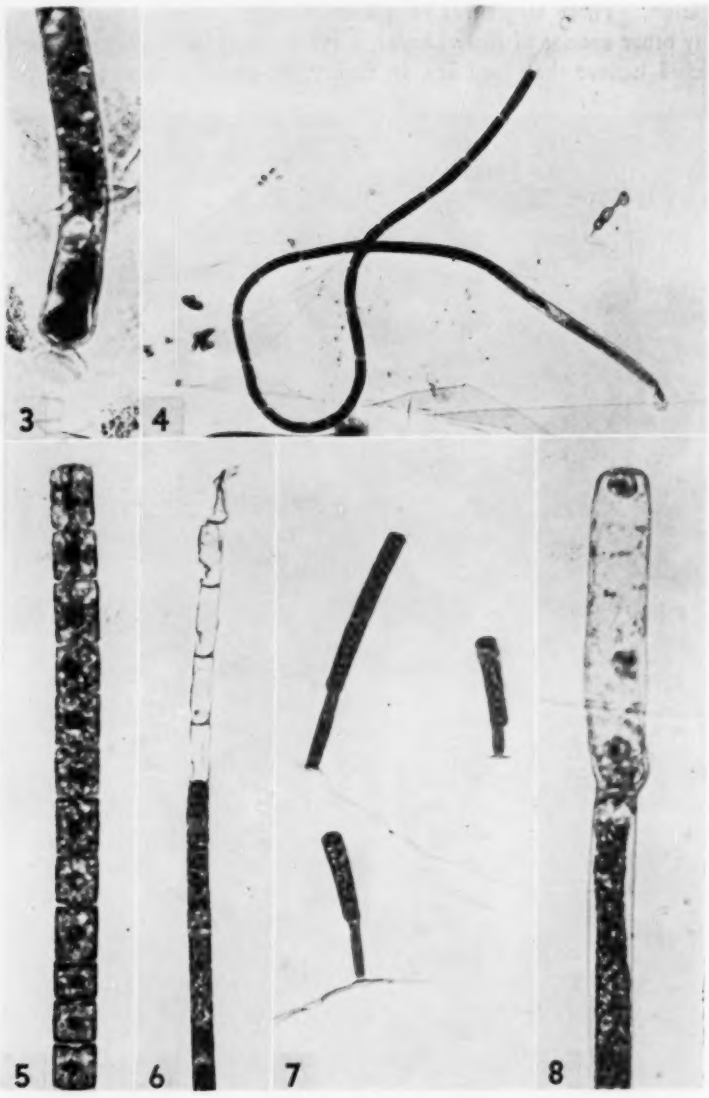
Uninucleate spores of type F (FIG. 5) have been observed to form in series up to 42 in one hypha. One thallus was found which contained 5 empty sporangia of spore type A, below which were forming spores of type F (FIG. 6). This unusual occurrence also has been found in *E. borariae* Lichtwardt (Lichtwardt, **1958**) and in *E. moniliformis.*

Small ellipsoid spores of type I were seen in various stages of ger-

mination. These structures of unknown origin, which I have seen in many other species of *Enterobryus,* serve as exogenous sources of infection. I believe that they are, in reality, uninucleate spores of type F



FIGS. 1, 2. *Enterobryus dixidesmi* from the milliped *Dixidesmus tallulanus.* 1. Coiled habit of thalli as seen in well-infected hosts, × 600. 2. Coils under lower magnification, × 160.



FIGS. 3–8.

FIGS. 3–8. *Enterobryus dixidesmi*. 3. Broad, disk-like holdfast, × 600. 4. Thallus with multinucleate sporangiospores (type A) and one empty terminal sporangium, × 160. 5. Row of uninucleate sporangiospores (type F), × 600. 6. Thallus with five empty sporangia of type A, subsequently producing uninucleate

that become morphologically modified some time during their transfer from one host to another or during their passage through the anterior digestive tract.

Enterobryus cherokiae Lichtwardt, sp. nov.

Thallis rectis vel laxe spiralibus, usque ad 7 mm long., 8–26 μ lat. Cytoplasmate granoso et in hyphis laterioribus vacuolato. Retinaculo plerumque cum lato orbi basali, sine caule elongate. Basi hyphae angustiori quo in loco retinaculo adjuncto. Sporangiosporis (typo A) 60–240 × 8–24 μ. Sporis multinucleatis (typo E) 60–83 × 15–30 μ.

Thalli straight or loosely spiraled, up to 7 mm long, 8–26 μ wide. Cytoplasm granular, and vacuolate in wider hyphae. Holdfast usually expanded at the base into a wide disk, without an elongated stalk; base of hypha narrowed at point of attachment to holdfast. Sporangiospores (type A) 60–240 × 8–24 μ. Multinucleate spores (type E) 60–83 × 15–30 μ.

Attached to the hindgut lining of the milliped *Cherokia georgiana* (Bollman). R. W. Lichtwardt slide H-l-e, TYPE, in author's collection.

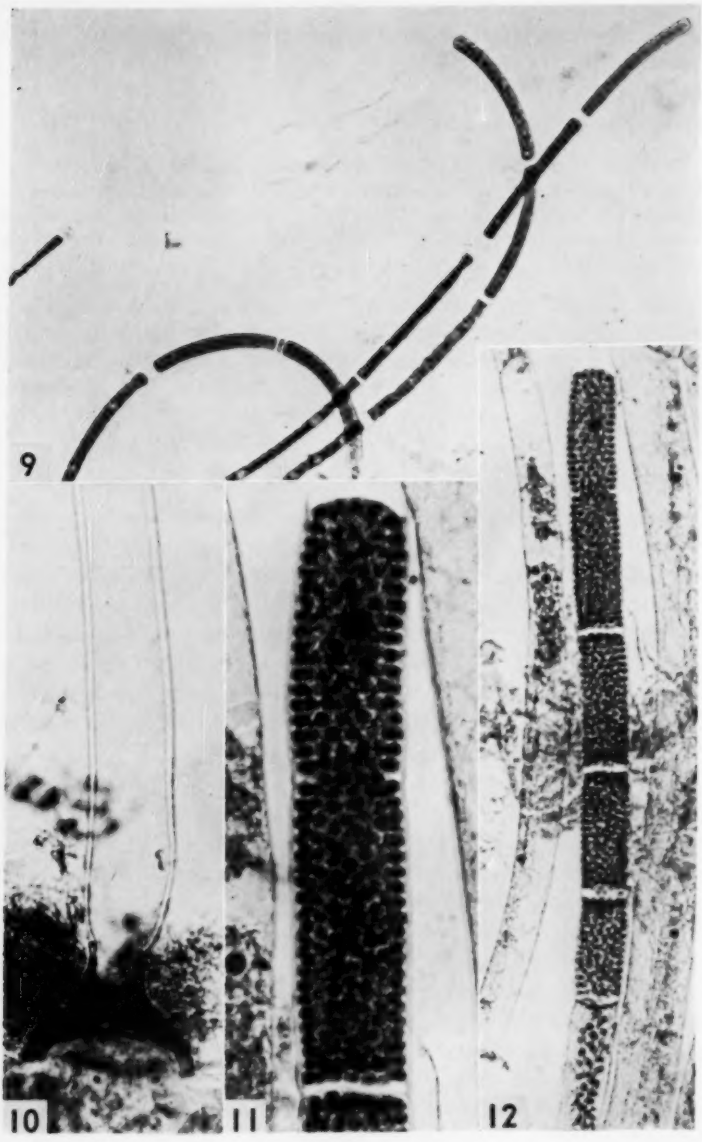
Cherokia georgiana is a relatively large, common and variable species of milliped in the southern Appalachian Mountains. The specimens for this study were collected around Highlands, N. C., and my collections were supplemented by a few individuals sent to me by Professor Leland Shanor and Mr. J. C. Knepton, Jr. Most of the adults that were dissected proved to be infected with *E. cherokiae*, some containing but a few thalli while others held so many thalli that the material was difficult to study due to its entanglement.

Enterobryus cherokiae is a rather large species (FIGS. 9–12), with hyphae often 5 to 7 mm in length and usually measuring 11–20 μ in width, though the range is greater, as indicated above. The thallus tips are abruptly rounded and occasionally curved as in *E. curyuri* Lichtwardt (Lichtwardt, **1954**).

The holdfast of *E. cherokiae* is very characteristic. The base of the holdfast consists of a disk, perhaps 45 μ wide on large hyphae, to which the hypha proper is almost directly attached. In the area of attachment the hypha narrows, as illustrated in FIG. 10. Smaller hyphae have proportionately smaller holdfasts, and may be slightly simpler in structure.

Spores of type A (FIG. 9) appear to fall into three size categories, depending upon the width of the hypha that produces them. Very nar-

spores, × 260. 7. Three germinating multinucleate spores; the new, elongating filaments form between the mother-spores and the holdfasts, × 260. 8. An apical mother-spore about to disintegrate, showing a cross-wall forming at its base, × 600.



FIGS. 9–12.

row hyphae have been observed, measuring about 8 μ in diameter, which produce very long, thin sporangiospores. They measure in length about 100–240 μ. Intermediate hyphae measuring about 10–15 μ in width produce spores about 60–70 μ long. Spores of these dimensions frequently have a slightly bulbous tip at the distal end, characteristic of spores of type A in many species of Eccrinales. Lastly, the wide hyphae, 19–26 μ in diameter, produce spores measuring about 140–240 μ in length. Spores of type A with intermediate dimensions also have been observed.

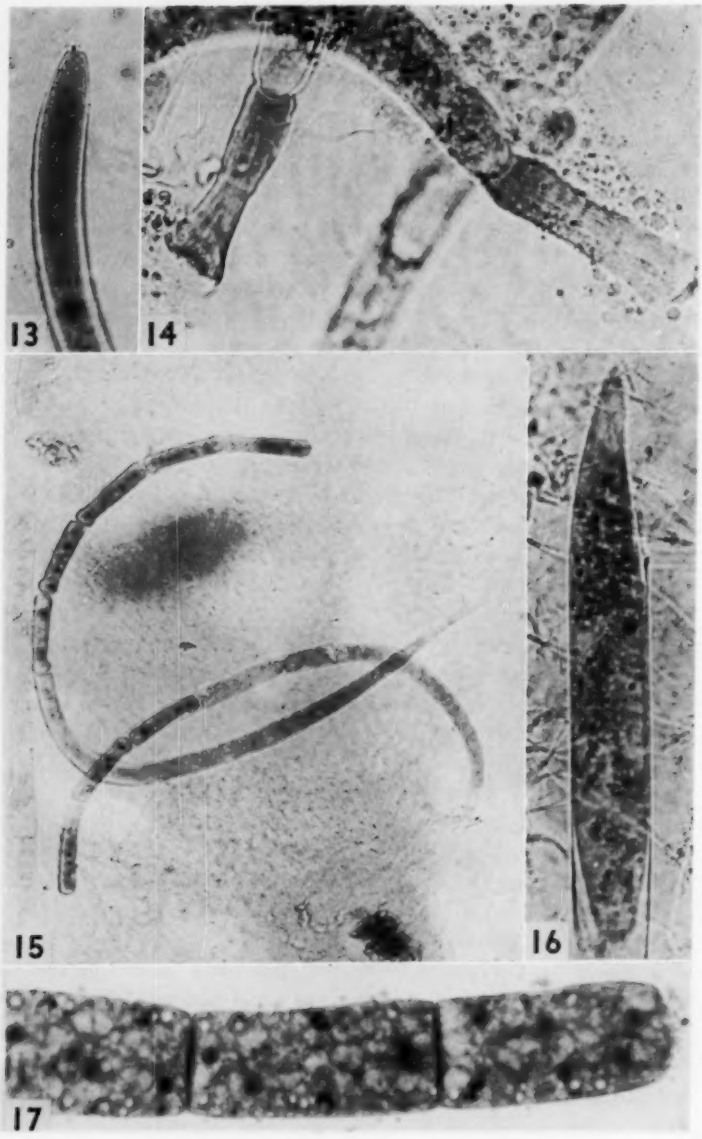
An unusual feature in type A spores of *E. cherokiae* is the variable number of nuclei which they may contain. These same spores in most other species of *Enterobryus* usually possess four or eight nuclei at the time they leave the sporangium. However, in *E. cherokiae* I have counted up to 24 nuclei in spores still within the sporangium.

Some host specimens contained many thalli producing multinucleate spores of type E (Lichtwardt, **1954**, **1958**), illustrated in FIGS. 11 and 12. These spores or cells of unknown function form by a migration of nuclei towards the apex, followed by cross-wall formation, resulting in a cell that is very densely packed with nuclei. A subsequent division of the nuclei within these cells may take place.

Tuzet, Manier, and Jolivet (**1957**, pp. 27–28) have questioned whether the spores I designated as type E are not in reality the same as spores of type A. However, the differences are not merely in the number of nuclei they contain, as the above authors seem to interpret. Rather, their ontogeny and structure both are markedly different, as my 1954 paper indicates.

One specimen of *Cherokia* contained in the posterior part of the hindgut a large number of small crook-shaped hyphae situated among the masses of filamentous bacteria which occupy this portion of the gut. They measured in width only about 3.5–5 μ, and in length up to about 190 μ. I have not determined whether these represent another phase of *Enterobryus cherokiae*, or whether they belong to another species (and genus?) of Eccrinales inhabiting the same host. I have seen hyphae very similar to the above in the posterior part of the hindgut of several individuals of *Narceus* (*Spirobolus*) *americanus* (Beauvois) that were infected at the same time with *Enterobryus elegans* Leidy.

FIGS. 9–12. *Enterobryus cherokiae* from the milliped *Cherokia georgiana*. 9. Thalli bearing spores of type A, × 260. 10. Holdfast, surrounded by bacteria, × 600. 11. Nuclei tightly packed within cells (spore type E); one cell contains a darkly-stained granule, × 600. 12. Lower magnification showing a row of cells, with nuclei aggregating beneath the last-formed cell, × 260.



FIGS. 13–17.

FIGS. 13–17. *Enterobryus ahlesi* from the milliped *Aphcloria montana*. 13 Hyphal tip, × 600. 14. Holdfasts, × 600. 15. Two thalli producing multinucleate

Enterobryus ahlesi Lichtwardt, sp. nov.

Thallis rectis, aut curvis propinquis basi, aliquando plus quam 1 cm long., plerumque 12–24 μ lat. Apice thallis sterilibus hebeti. Cytoplasmate hyalino. Retinaculo saepius sine orbi basali, usque ad 60 μ long. Sporangiosporis (typo A) plerumque 43–160 × 7–14 μ. Sporis reticulatis (typo A?) 32–62 × 20–35 μ.

Thalli straight, or curved near the base, occasionally exceeding 1 cm in length, usually 12–24 μ wide. Tips of vegetative thalli bluntly pointed. Cytoplasm hyaline. Holdfast usually without a basal disk, up to 60 μ long. Sporangiospores (type A) usually 43–160 × 7–14 μ. Vacuolate spores (type A?) 32–62 × 20–35 μ.

Attached to the hindgut lining of the milliped *Apherloria montana* (Bollman). R. W. Lichtwardt slide T-l-1a, TYPE, in author's collection.

The species is named after Mr. Harry E. Ahles who collected the host millipeds, and who has brought other species of millipeds to me on numerous occasions for dissection.

Two adult male specimens of the large, handsome *Apheloria montana* were collected in Claiborne County, Tennessee. One was heavily infected with *Enterobryus*, the other moderately so. Despite the limited number of hosts available, the fungus appears to be sufficiently distinct to warrant describing it as a new species.

The hyphae of *E. ahlesi* generally are very long, and they are attached throughout the hindgut, but concentrated mostly near the anterior portion. The tips of the vegetative thalli in most cases are bluntly pointed (FIG. 13) as in *E. apheloriae* Lichtwardt from *Apheloria iowa* Chamberlin, but, unlike this species, the thalli of *E. ahlesi* are not tapered towards the holdfast.

The holdfasts average about 12 μ in diameter, and their length varies from 6–60 μ, the longer holdfasts showing longitudinal striations (FIG. 14). There were no basal disks on most holdfasts, although an exception occurs in certain hyphae described below.

Typical spores of type A have two forms. The first is a relatively short spore with a bulbous base at the proximal end (FIG. 15), measuring about 45–70 × 7–10 μ. The second form is considerably longer, usually about 160 μ in length and 11–14 μ in width. The latter has a bulbous distal tip, indicating that it escapes from the distal end of the sporangium.

One of the specimens dissected had two thalli producing what appears to be a modified form of spore type A. These thin-walled spores were

spores of type A, × 260. 16. A fusiform spore of type A emerging from the terminal end of the thallus, × 600. 17. Large, vacuolate spores with peripheral nuclei, common in this species, × 600.

fusiform, pointed at the distal tip, and somewhat rounded at the proximal end (FIG. 16). Each was borne within its own sporangium, with the very thin end-walls following the contour of the proximal end of the spore. At the time the material was fixed, some of the spores were emerging by passing through the free end of the hypha. Their method of germination was not followed. The thalli contained a total of twelve such spores, and both filaments were attached to the gut lining by means of a holdfast with a broad, basal disk that looked very much like the holdfast of *E. cherokiae* (FIG. 10). These thalli were sufficiently different from the others in *Apheloria montana* that one might question whether they represent the same species.

A large proportion of the hyphae were bearing spores or cells of a large and vacuolate type (FIG. 17). The nuclei in these were located near the periphery, usually with a maximum of eight nuclei per cell. The actual function of such cells is not known, though I have occasionally observed similar cells in other species of *Enterobryus*.

SUMMARY

Three new species of *Enterobryus* (Eccrinales) are described that inhabit the hindguts of three millipeds from southeastern United States. They are *E. dixidesmi* in *Dixidesmus tallulanus* Chamberlin, *E. cherokiae* in *Cherokia georgiana* (Bollman), and *E. ahlesi* in *Apheloria montana* (Bollman).

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A CORRELATION OF INTERSPECIFIC FERTILITY AND CONIDIAL MORPHOLOGY IN SPECIES OF HELMINTHOSPORIUM EXHIBITING BIPOLAR GERMINATION [1]

R. R. NELSON [2]

(WITH 1 FIGURE)

It is generally recognized that species of the Fungi Imperfecti are classified in genera and the genera further grouped into families, virtually without "natural" criteria. This concept is best illustrated by the fact that frequently a single genus of Ascomycetes may have conidial stages in various imperfect genera. In addition, a single genus of the Imperfecti may have species associated with a variety of ascomycete genera. Furthermore, it is recognized that spore characteristics of size, shape, and number of septations are extremely variable and may vary widely in response to change in light, temperature, substrate, age, and other factors. Thus, it would appear that classification of species in the Fungi Imperfecti on the basis of conidial morphology is largely a matter of convenience and functions as a temporary disposition until conidial forms are associated with sexual stages.

Of the species of *Helminthosporium* 13 exhibiting bipolar germination have ascigerous stages in *Cochliobolus, Helminthosporium turcicum* Pass. has its perfect stage in *Trichometasphaeria, Helminthosporium rostratum* Drechs. has been reported (1) to have a perfect stage similar to that described for *H. turcicum,* and the remaining have not been associated with perfect stages. In spite of an apparent lack of "natural" criteria in placing species in the genus *Helminthosporium,* the association of at least 13 *Helminthosporium* species with *Cochliobolus* suggests that classification on the basis of conidial morphology and other asexual characters may not be totally invalid for this group.

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The author has been concerned with the evolution of related *Helminthosporium* species. The ultimate objective in these investigations is to determine the maximum genetic potential or near potential for pathogenicity in species attacking gramineous hosts. If related species have evolved along parallel lines, it is likely that basic mechanisms controlling pathogenicity are at least partly similar. A knowledge of the kind and magnitude of genetic similarities and of genetic differences in other basic mechanisms, including patterns of sexuality, is equally important. In *Cochliobolus sativus* (Ito & Kurib.) Drechs. (*Helmintho-*

TABLE I

SPECIES OF HELMINTHOSPORIUM USED IN INTERSPECIFIC CROSSES

Species		Number of isolates used	Host
Conidial stage	Perfect stage		
H. carbonum	*Cochliobolus carbonum*	32	Corn
H. maydis	*C. heterostrophus*	57	Corn
H. oryzae	*C. miyabeanus*	21	Rice
H. sativum	*C. sativus*	24	Barley
H. victoriae	*C. victoriae*	22	Oats
H. setariae	*C. setariae*	2	Millet
H. sorghicola	Not known	3	Johnson grass
H. sacchari	Not known	3	Sugar cane
H. leersii	Not known	3	Cutgrass
H. sp. 1	Not known	1	Rice
H. sp. 2	Not known	1	Rice
H. sp. 3	Not known	1	[a]
H. sp. 4	Not known	1	Bermuda grass
H. sp. 5	Not known	1	Bermuda grass
H. sp. 6	Not known	1	Barley
H. sp. 7	Not known	1	Fescue

[a] = isolated from soil.

sporium sativum Pam., King & Bakke) (**7**), *Cochliobolus heterostrophus* Drechs. (*Helminthosporium maydis* Nisik. & Miyake) (**3**), *Cochliobolus carbonum* Nelson (*Helminthosporium carbonum* Ullstrup) (**4**), *Cochliobolus nodulosus* Luttrell (*Helminthosporium nodulosum* Berk. & Curt.) (**2**), and *Cochliobolus victoriae* Nelson (*Helminthosporium victoriae* Meehan & Murphy) (**6**), compatibility or mating type is controlled by a single major gene locus. Variability in degree of compatibility has been demonstrated in some species and observed in others, indicating that additional genes influence mating capacity. It was apparent that similarities and differences in the sexual mechanisms of these and related species would be reflected in the success or failure of attempted interspecific crosses. Interspecific fertility between certain isolates of *Hel-*

minthosporium species was reported recently (**5**). This paper summarizes available data on the extent and degree of interspecific fertility and correlates conidial morphology and interspecific compatibility.

METHODS AND MATERIALS

The 16 species of *Helminthosporium* used in interspecific crosses, as well as the number of isolates of each species, are presented in TABLE I. Whenever possible, isolates of each species were obtained from several geographic areas in an effort to obtain broad genetic bases. Six of the species have perfect stages in *Cochliobolus*, 3 are described species with no known perfect stage, and 7 are unidentified but considered to

TABLE II

A GROUPING OF THE SPECIES OF HELMINTHOSPORIUM USED IN INTERSPECIFIC CROSSES ON THE BASIS OF CONIDIAL MORPHOLOGY

Species and morphology class[a]				
1	2	3	4	5
H. maydis *H. oryzae* *H. sacchari* *H. leersii* *H. sorghicola* *H.* sp. 1[b] *H.* sp. 2 *H.* sp. 3	*H.* sp. 4	*H. carbonum* *H. victoriae* *H. setariae*	*H. sativum*	*H.* sp. 5 *H.* sp. 6 *H.* sp. 7

[a] Morphology classes 1–5 correspond to classes shown in FIG. 1.
[b] Unidentified species are the same as listed in TABLE I.

be distinct morphologically from other species used. All 16 species are heterothallic and exhibit bipolar germination. For purposes of correlation, the 16 species were placed in 5 classes, based on shape and size of conidia (TABLE II). The morphology classes are depicted in FIG. 1. The author recognizes that size and shape of conidia of most bipolar species of *Helminthosporium* vary to a considerable extent and that the limits encountered for these morphological characters frequently overlap between closely related species. The 5 classes, illustrated by photomicrographs of representative species, are intended only to characterize the species in gross morphology. The marked variation in conidial morphology frequently makes it extremely difficult to determine whether a specific isolate is representative of one species or a closely related species. For the most part, this difficulty is encountered only in classifying species within any given morphology class. For example, in the

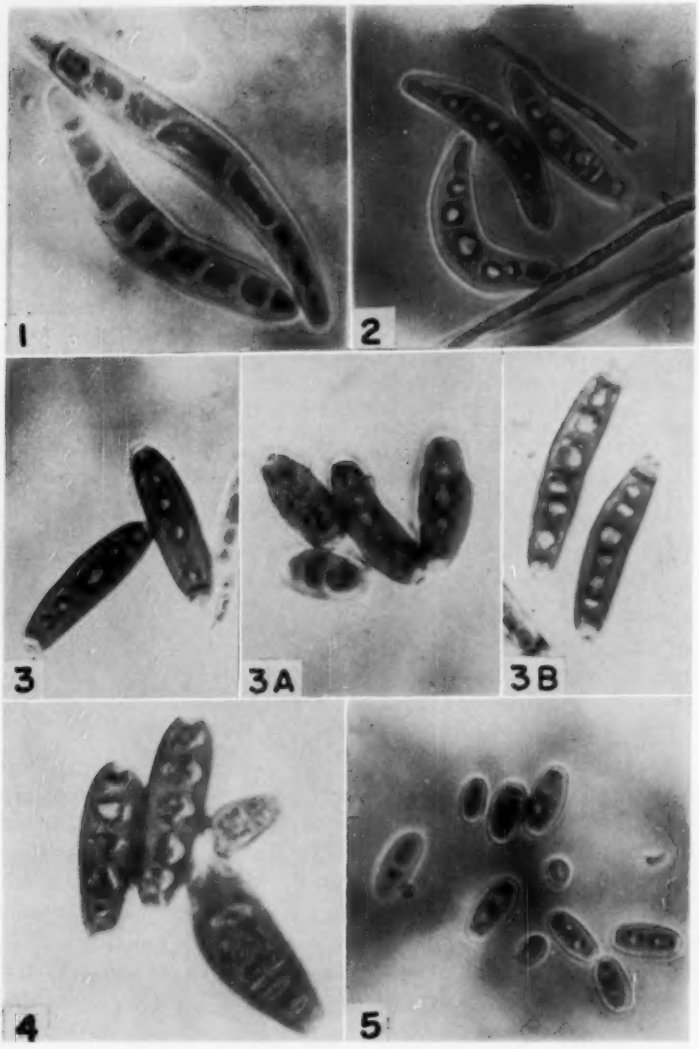

FIG. 1. Conidial morphology classes 1–5 represented by the following species: group 1 by *Helminthosporium oryzae;* group 2 by *H.* sp. 4 (see Table I); group 3 by *Helminthosporium victoriae* (3 and 3A) and *Helminthosporium carbonum* (3B); group 4 by *Helminthosporium sativum;* group 5 by *H.* sp. 7 (see Table I).

absence of host reaction, it would be a calculated risk to attempt to classify most isolates of *H. maydis* and *Cochliobolus miyabeanus* (Ito & Kurib.) Drechs. (*Helminthosporium oryzae* Breda de Haan) as either species. However, it would be no problem to determine whether an isolate was *H. maydis* or *H. victoriae*. Furthermore, it would be relatively simple to determine in which of the 5 morphology classes any isolate of any of the species studied should be placed. Based on these concepts, the arbitrary erection of these classes appears feasible and justified.

The mating techniques used were similar to those described previously (3). Interspecific pairings were made by placing small pieces of mycelium on opposite sides of a section of sterile corn leaf in the center of a Petri plate containing Sachs' nutrient agar. All pairings were incubated continuously at 24° C.

RESULTS AND DISCUSSION

Of the possible 120 crosses of the 16 species in all combinations, 13 produced viable ascospores and 14 developed sterile asci. In some of the crosses producing sterile asci, all asci exhibited normal morphological development and were indistinguishable from fertile asci containing ascospores. In other crosses, some of the asci were poorly developed and never attained normal size or shape. None of the crosses produced only poorly developed, abnormal asci. In each of the 27 crosses, all isolates of one species were paired with all isolates of the other species one or more times. The production of sterile asci in crosses of heterothallic species demonstrates a definite genetic compatibility, in that plasmogamy, crozier formation, normal morphological development of the ascus, and, in all crosses thus far examined, the formation of a diploid nucleus have occurred. Aborted ascospores were observed in certain of the crosses producing sterile asci, whereas no evidence of ascospore delimitation was observed in other similar crosses. The fact that spore formation occurs after the mitotic division suggests the ascospore abortion may be genetically controlled. Furthermore, in crosses in which no spore formation was evident, it is likely that the complete meiotic sequence did not occur.

Of the possible 34 crosses between species with similar conidial morphology (crosses within classes 1, 3, and 5), 10 produced ascospores and 2 produced sterile asci (TABLE III). Certain of the fertile crosses produced some ascospores which were poorly developed and nonviable, while other fertile crosses produced only normal ascospores. Data on

the relative frequency of aborted ascospore production were not obtained. The number of different crosses between isolates of 2 similar species ranged from 1–1197 and totaled 2707, whereas the number of attempted crosses, including repeated pairings, ranged from 1–3221 and totaled 7603. The data in TABLE III do not include crosses attempted for confirming or repeating a previously fertile cross. Certain of the crosses between isolates were obtained in the first mating, while others occurred as a result of repeated attempts. Furthermore, each of the 46 crosses

TABLE III

CROSSES BETWEEN SPECIES OF HELMINTHOSPORIUM WITH SIMILAR CONIDIAL MORPHOLOGY

Cross	Farthest stage of development	Number of crosses		
		Different	Attempted[a]	Successful[b]
H. maydis × *H. oryzae*	ascospores	1197	1712	2
H. maydis × *H. sorghicola*	ascospores	171	430	2
H. maydis × *H. leersii*	ascospores	171	513	1
H. maydis × *H.* sp. 1[c]	ascospores	57	285	1
H. maydis × *H.* sp. 2	ascospores	57	285	2
H. maydis × *H.* sp. 3	ascospores	57	228	1
H. sp. 1 × *H.* sp. 3	ascospores	57	228	1
H. sp. 2 × *H.* sp. 3	ascospores	1	1	1
H. sp. 1 × *H.* sp. 2	ascospores	1	1	1
H. carbonum × *H. victoriae*	ascospores	704	3221	31
H. maydis × *H. sacchari*	asci	171	384	2
H. oryzae × *H. sacchari*	asci	63	315	1
	Total	2707	7603	46

[a] Attempted crosses include 2 or more pairings of 2 isolates.
[b] Numbers of successful crosses do not include repeated crosses between 2 isolates.
[c] Unidentified species are the same as listed in TABLE I.

represents different crosses between isolates and does not include repeated crosses of compatible isolates although all crosses have been successfully repeated at least once.

Of the possible 87 crosses between species with different conidial morphology, 3 produced ascospores and 12 produced sterile asci. Ascospore production in crosses of *H. carbonum* × *H. maydis*, *H. carbonum* × *H.* sp. 1, and *H. carbonum* × *H.* sp. 3 was scant and approximately 80 per cent of these did not germinate. The inability to obtain substantial numbers of ascospore isolates has precluded analysis of the inheritance of spore morphology. The number of different crosses between isolates of 2 morphologically distinct species ranged from 22–1824 and totaled 5417, whereas the number of attempted crosses, including repeated pairings, ranged from 88–3160 and totaled 10,235 (TABLE IV).

Seventy-one different crosses were obtained from a total of 17,838 attempted matings, representing a relative frequency of .39 per cent. Frequencies of single interspecific crosses are not meaningful in view of the differences in numbers of isolates of the species. It is apparent that success in genetic studies such as these depends primarily on using large numbers of isolates from diverse geographic areas. Eighteen of the 27 interspecific crosses involved *H. maydis* or *H. carbonum*, and

TABLE IV

CROSSES BETWEEN SPECIES OF HELMINTHOSPORIUM WITH DIFFERENT CONIDIAL MORPHOLOGY

Cross	Farthest stage of development	Number of crosses		
		Different	Attempted[a]	Successful[b]
H. maydis × *H. carbonum*	ascospores	1824	3160	2
H. carbonum × *H.* sp. 1[c]	ascospores	32	160	1
H. carbonum × *H.* sp. 3	ascospores	32	160	1
H. carbonum × *H. sativum*	asci	768	1118	3
H. victoriae × *H. sativum*	asci	528	1112	2
H. setariae × *H. sativum*	asci	48	144	1
H. maydis × *H. setariae*	asci	114	342	2
H. maydis × *H. sativum*	asci	1368	2104	2
H. oryzae × *H. sativum*	asci	504	1012	1
H. maydis × *H.* sp. 4	asci	57	228	2
H. carbonum × *H.* sp. 7	asci	32	167	2
H. carbonum × *H.* sp. 4	asci	32	160	2
H. carbonum × *H.* sp. 5	asci	32	160	1
H. sativum × *H.* sp. 1	asci	24	120	1
H. victoriae × *H.* sp. 6	asci	22	88	2
	Total	5417	10,235	25

[a] Attempted crosses include 2 or more pairings of 2 isolates.
[b] Numbers of successful crosses do not include repeated crosses between 2 isolates.
[c] Unidentified species are the same as listed in TABLE I.

89 of the 174 isolates used were of these 2 species. It is likely that as additional isolates of other species are obtained, other interspecific crosses will be obtained.

Studies on the compatibility reaction of ascospore isolates from crosses demonstrated that some were cross-fertile with one or the other of the parental isolates, some did not mate with either parent, and a very few were compatible with both. Progeny cross-fertile with both parental isolates, as well as those exhibiting complete backcross sterility, more than likely represent recombinant classes. Further studies on the phenomena of "dual" compatibility and sterility are in progress.

The grouping of the 16 species into 5 arbitrary morphology classes appears to have had justification. The incidence of interspecific crosses

was markedly higher between species of the same group than between species of different groups. It is not possible to discuss the relative frequency of crossing among the different groups as classes 1–5 are not intended to represent a sequential pattern of morphology types. It must be assumed that gross morphological differences are genetically controlled. Consequently, it is reasonable to assume that species differing in genes conditioning morphology also differ in genes controlling other factors. These studies suggest that the magnitude of such differences is greater among species in different morphology classes rather than among species within any specific class.

The question could be raised as to whether species that are similar morphologically and produce either viable ascospores or sterile asci when crossed are valid species or merely variants of a single species. It is true that many species of phytopathogenic fungi have been described solely on the basis of host reaction. These studies indicate that morphologically similar species differ in one or more genes conditioning sexual reproduction. Although genetic similarities or differences in sexual mechanisms may not serve as convenient as criteria for classification, they provide a valid, perhaps even a "natural," basis for differentiation. On the basis of degree of interspecific fertility, *H. carbonum* and *H. victoriae* appear to be closely related. However, only 31 of 3221 attempted crosses of isolates of these species were fertile, less than one per cent. If *H. carbonum* and *H. victoriae* were considered as one species, it would be necessary to propose a complex system of sterility and inhibitory genes to account for more than 99 per cent cross-sterility among isolates.

All interspecific crosses obtained in these studies produced a *Cochliobolus* sexual stage. It is likely, therefore, that the species of *Helminthosporium* used in these studies that have no known perfect stage probably have perfect stages in *Cochliobolus*.

These studies suggest that species of *Helminthosporium* exhibiting bipolar germination with notable exceptions, may have one or more "natural" criteria in common. Perhaps additional differentiating characters are present within this group. All attempts to cross isolates of *H. turcicum* and *H. rostratum* with other bipolar species have been unsuccessful. In addition to having perfect stages in *Trichometasphaeria* or a similar genus, both *H. turcicum* and *H. rostratum* have conidia with a protuberant hilum, a character not present in the bipolar species used in the present studies. It is probable that as sexual stages of additional species of *Helminthosporium* are obtained, a more accurate approach to classification within this heterogeneous group may be available.

SUMMARY

Of 8124 attempted interspecific crosses between 174 isolates of 16 species of *Helminthosporium* exhibiting bipolar germination, 47 produced viable ascospores and 24 produced sterile asci. The 16 species were placed into 5 morphology classes, based on relative shape and size of conidia. Forty-six of 2707 attempted crosses between species with similar conidial morphology were fertile, while 25 of 5417 crosses between species with different morphology were fertile. Although species in the Fungi Imperfecti are considered to be classified without "natural" criteria and merely as a matter of convenience, the frequency of cross fertility between species with a similar or different conidial morphology indicates that genetic similarities exist between related conidial forms.

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FURTHER INVESTIGATIONS ON THE PRESERVATION OF MOLDS

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Various methods have been used for preserving fungi and other microorganisms. This paper gives further evidence of the value of the procedures now in use, particularly that of lyophilization. Three reports have already appeared from this laboratory, Raper and Alexander (**1945**), Fennell et al. (**1950**) and Mehrotra and Hesseltine (**1958**). As a continuation, all species of fungi except the Aspergilli, the Penicillia and the yeasts, that have been in the ARS Culture Collection for a long time, were tested for viability. Also, some Actinomycetes received and lyophilized prior to 1949 were examined. Most of the cultures have been in lyophil for 17 years. Some Mucorales that had been preserved in 1945 by the mineral oil overlay method were also tested. Additional cultures of Mucorales on dried slants were covered with warm melted agar. This study was to test viability and to see if morphology had remained constant.

MATERIALS AND METHODS

Lyophilized cultures of 363 strains of Mucorales, Entomophthorales, Ascomycetes, Fungi Imperfecti and Actinomycetes were tested for viability as follows: The lyophil tube, along with malt broth tubes, were kept at room temperature for 1 to 2 days, were then opened and each pellet was dissolved in 0.6 ml of 2% malt extract. In most cases, the resulting spore suspension was streaked on two Petri dishes containing potato dextrose agar, prepared according to Haynes et al. (**1955**). The Actinomycetes were streaked on tomato paste-oatmeal agar (formula of Pridham et al., **1956–1957**). *Kernia brachytricha* (Ames) Benj. was streaked on yeast extract agar, as prepared by Haynes et al. (**1955**). Except for the Actinomycetes, when the lyophil tubes were opened, the same strains were also transferred from slants to plates of potato dextrose agar or corresponding medium.

Most of the plate cultures were incubated at 26° C and were examined for viability at 4 to 10 days, depending on the rate of growth. In a few instances a different temperature was required, e.g., the Actinomycetes were incubated at 28° C, *Dactylomyces thermophilus* Sopp at 37° C and *Thamnidium elegans* Link ex Gray at 15° C.

TABLE I

VIABILITY OF LYOPHILIZED PREPARATIONS OF SELECTED FUNGUS CULTURES

Name	NRRL no.	Date processed	Approx. age in years	Viability*
MUCORALES				
Absidia blakesleeana Lendn.	1300	3/24/42	17	++++
A. butleri Lendn.	1307	3/24/42	17	+++
A. butleri Lendn.	1340	3/27/42	17	+++
A. butleri Lendn.	1462	3/24/42	17	++++
A. butleri Lendn.	1709	6/25/42	17	+++
A. capillata v. Tiegh.	1309	3/24/42	17	++++
A. coerulea Bainier	1310	3/24/42	17	++++
A. coerulea Bainier	1314	3/24/42	17	++++
A. cylindrospora Hagem	1316	3/24/42	17	++++
A. dubia Bainier	1318	3/24/42	17	++++
A. glauca Hagem	1324	3/25/42	17	++++
A. hyalospora (Saito) Lendn.	1330	3/25/42	17	++++
A. lichtheimi Lendn.	1331	3/25/42	17	++++
A. ramosa (Lindt) Lendn.	1590	6/1/42	17	++++
A. ramosa (Lindt) Lendn.	1592	6/1/42	17	++++
A. ramosa (Lindt) Lendn. var. *ramosa* Lendn.	1332	3/25/42	17	++++
A. ramosa (Lindt) Lendn. var. *rasti* Lendn.	1333	3/25/42	17	++++
A. ramosa (Lindt) Lendn. var. *zurcheri* Lendn.	1334	3/25/42	17	++++
A. regnieri (Luc. & Cost.) Lendn.	1335	3/25/42	17	++++
A. repens v. Tiegh.	1336	3/25/42	17	+++**
A. spinosa Lendn.	1347	3/27/42	17	++**
A. "whorled"	1341	3/25/42	17	++++
A. sp.	1516	3/9/42	17	+++
A. sp.	1561	6/8/42	17	++++
A. sp.	1807	4/24/42	17	++++
Actinomucor elegans (Eidam) C. Benj. & Hesselt.	685	5/19/42	17	++++
A. elegans (Eidam) C. Benj. & Hesselt.	1706	5/22/42	17	++++
Blakeslea trispora Thaxt.	1348	5/14/46	13	++++
B. trispora Thaxt.	1718	1/5/51	8	++++**
Chaetocladium brefeldii v. Tiegh. & Le Mon.	1349	10/22/51	8	+++
Circinella minor Lendn.	1353	3/25/42	17	++++
C. mucoroides Saito	1354	3/25/42	17	++++
C. muscae (Sorok.) Berl. & de T.	1355	6/17/42	17	++++
C. muscae (Sorok.) Berl. & de T.	1356	3/25/42	17	++++
C. muscae (Sorok.) Berl. & de T.	1364	3/26/42	17	++++
C. muscae (Sorok.) Berl. & de T.	1367	3/27/42	17	++++
C. umbellata v. Tiegh. & Le Mon.	1351	3/25/42	17	++++
C. umbellata v. Tiegh. & Le Mon.	1352	3/25/42	17	++++
C. umbellata v. Tiegh. & Le Mon.	1594	6/1/42	17	++++
Coemansia brasiliensis Thaxt.	1566	6/8/42	17	+++
C. guatemalensis Thaxt.	1565	6/10/42	17	++
C. pectinata Bainier	1564	12/17/45	14	+
Cunninghamella blakesleeana Lendn.	1368	4/11/46	13	+**
C. blakesleeana Lendn.	1369	3/27/42	17	+++
C. bertholletiae Stahel	1375	3/27/42	17	++++**
C. dalmatica Pispek	1394	4/17/46	13	++++
C. echinulata (Thaxt.) Thaxt.	1382	3/27/42	17	++++
C. echinulata (Thaxt.) Thaxt.	1384	3/27/42	17	++++

* ++++ = excellent viability; +++ = good viability; ++ = fair viability; + = poor viability; − = no growth.

** More than one tube opened.

TABLE I—*Continued*

Name	NRRL no.	Date processed	Approx. age in years	Viability*
C. elegans Lendn.	1388	3/30/42	17	+
C. polymorphia Pispek	1395	3/30/42	17	++++
Gilbertella persicaria (Eddy) Hesselt.	1556	6/5/42	17	++++
Helicostylum piriforme Bainier	1396	3/30/42	17	++++
H. piriforme Bainier	1399	3/30/42	17	++++
H. piriforme Bainier	1591	6/1/42	17	++++
Mortierella isabellina Oud.	1757	4/15/42	17	+++
M. sp.	1458	4/1/42	17	++++
M. sp.	1617	6/9/42	17	−
M. sp.	1707	6/2/42	17	++++
Mucor caninus Pers. ex Fr.	1424	4/1/42	17	++++
Mucor caninus Pers. ex Fr.	1425	4/19/46	13	++++
M. circinelloides v. Tiegh.	1405	3/30/42	17	++++
M. dubius Wehmer	1406	3/31/42	17	++++
M. genevensis Lendn.	1407	3/31/42	17	++++
M. genevensis Lendn.	1758	4/15/42	17	++++
M. genevensis Lendn.	1412	3/31/42	17	++++
M. genevensis Lendn. Mutant A	1408	3/31/42	17	+++
M. genevensis Lendn. Dwarf	1409	3/31/42	17	+
M. globosus A. Fisch.	1759	4/15/42	17	++++
M. griseo-cyanus Hagem	1413	3/31/42	17	++++
M. hiemalis Wehmer	1419	3/31/42	17	+++
M. hiemalis Wehmer var. *albus* Lendn.	1420	3/31/42	17	++++
M. javanicus Wehmer	1421	3/31/42	17	++++
M. lamprosporus Lendn.	1422	4/1/42	17	++++
M. microsporus Namyslowski	1423	4/6/42	17	++++
M. plumbeus Bon.	687	5/19/42	17	++++
M. pusillus Lindt	1426	6/22/42	17	++++**
M. racemosus Fres.	1427	4/1/42	17	++++
M. racemosus Fres.	1688	6/16/42	17	++++
M. ramannianus Moell.	1454	4/7/42	17	++++
M. ramannianus Moell.	1559	6/5/42	17	++++
M. rouxii (Calm.) Wehmer	1429	4/24/46	13	++++
M. rouxii (Calm.) Wehmer	1894	12/22/47	12	++++
M. silvaticus Hagem	1431	4/1/42	17	++++
M. sphaerosporus Hagem	1436	4/15/42	17	++++
M. tenuis Bainier	1435	4/6/42	17	++++
M. varians Povah	1760	4/15/42	17	++++
M. sp. (strain III)	1438	6/22/42	17	++++
M. sp. (strain IV)	1442	4/24/42	17	++++
M. sp. (strain VII)	1452	6/17/42	17	++++
M. sp.	1456	4/7/42	17	++++
M. sp.	1533	2/10/42	17	++++
M. sp.	1545	3/11/42	17	++++
Mycotypha microspora Fenner	684	3/24/42	17	++++
Parasitella parasitica (Bainier) Sydow	1459	3/12/42	17	++++
Phascolomyces articulosus Boed.	1593	6/1/42	17	+
Phycomyces blakesleeanus Burgeff	1464	5/6/46	13	+++
P. blakesleeanus Burgeff	1465	12/7/45	14	++++
P. blakesleeanus Burgeff	1554	3/11/42	17	++++
P. blakesleeanus Burgeff	1555	3/11/42	17	++++
P. nitens Kunze	1467	4/1/42	17	++++
Rhizopus arrhizus Fisch.	1469	4/1/42	17	++++
R. arrhizus Fisch.	1470	4/1/42	17	++++
R. arrhizus Fisch.	1702	6/22/42	17	++++

TABLE I—*Continued*

Name	NRRL no.	Date processed	Approx. age in years	Viability*
R. "Boullard"	1891	11/5/42	17	++++
R. chinensis Saito	1471	4/7/42	17	++++
R. cohnii (Cohn apud Lichtheim) Berl. et de T.	1517	3/9/42	17	++++
R. delemar (Boid.) Wehmer & Hanz.	1472	4/25/46	13	++++
R. japonicus Vuill.	1895	12/31/42	17	++++
R. liquefaciens Yamazaki	1512	3/9/42	17	++++
R. microsporus v. Tiegh.	1508	3/9/42	17	++++
R. microsporus v. Tiegh.	1577	6/22/42	17	++++
R. nodosus Namyslowski	1473	4/7/42	17	++++
R. nodosus Namyslowski	1474	4/7/42	17	++++
R. oryzae Went et Geerl.	395	3/11/42	17	++++
R. oryzae Went et Geerl.	1526	3/10/42	17	++++
R. oryzae Went et Geerl.	1528	3/10/42	17	++++
R. Peka I Takeda	1522	3/10/42	17	++++
R. reflexus Bainier	1475	4/7/42	17	++++
R. shanghaiensis Yamazaki	1518	3/9/42	17	++++
R. stolonifer (Ehrenb. ex Fr.) Vuill.	1259	5/8/42	17	++++
R. stolonifer (Ehrenb. ex Fr.) Vuill.	1477	4/7/42	17	++++
R. stolonifer (Ehrenb. ex Fr.) Vuill.	1478	4/7/42	17	++++
R. stolonifer (Ehrenb. ex Fr.) Vuill.	1483	4/8/42	17	++++
R. stolonifer (Ehrenb. ex Fr.) Vuill.	1484	4/8/42	17	++++
R. suinus Nielson	1557	6/5/42	17	++++
R. tonkinensis Vuill.	1896	12/31/42	17	++++
R. tritici Saito	1476	4/7/42	17	++++
R. sp.	1515	3/9/42	17	++++
R. sp.	1530	3/10/42	17	++++
R. sp.	1534	2/10/42	17	++++
R. sp.	2004	9/27/45	17	++++
Syncephalastrum racemosum Schroet.	1506	3/9/42	17	++++
S. racemosum Schroet.	1485	4/7/42	17	++++
S. sp.	1507	3/9/42	17	++++
S. sp.	1623	6/4/42	17	++++
Thamnidium elegans Lk. ex S. F. Gray	1613	6/1/42	17	+++
Zygorhynchus heterogamus (Vuill.) Vuill.	1489	6/30/53	6	+**
Z. heterogamus (Vuill.) Vuill.	1616	6/1/42	17	+++
Z. moelleri Vuill.	1493	6/2/42	17	+
Z. moelleri Vuill.	1496	6/2/42	17	+
Z. moelleri Vuill.	1498	6/2/42	17	++++
Z. moelleri Vuill.	1499	6/2/42	17	−
Z. moelleri Vuill.	1500	6/2/42	17	++++
Z. moelleri Vuill.	1625	5/22/42	17	++++
Z. moelleri Vuill.	1763	4/17/42	17	+++
Z. moelleri Vuill.	1497	6/2/42	17	++++
ENTOMOPHTHORALES				
Conidiobolus sp.	1612	12/22/47	12	−
Delacroixia coronata (Cost.) Sacc. & Sydow	1912	3/27/43	16	−
Entomophthora apiculata Thaxt.	1626	12/22/47	12	−
ASCOMYCETES				
Arachniotus aureus (Eidam) Schroet.	1902	3/10/43	16	+++
Anthostomella sp.	1865	9/28/42	17	+++
Byssochlamys fulva Olliver & G. Smith	1125	6/22/42	17	++++
B. nivea Westl.	2205	5/18/49	10	++
B. sp.	2225	11/16/49	10	++++
Ceratocystis adiposa (Butl.) Moreau	1801	3/27/42	17	++++
C. fimbriata Ell. & Halst.	1802	3/27/42	17	++

TABLE I—*Continued*

Name	NRRL no.	Date processed	Approx. age in years	Viability*
Chaetomium caprinum Bainier	1867	9/28/42	17	+++
C. cochliodes Palliser	2170	3/22/49	10	+
C. dolichotrichum Ames	1606	6/9/42	17	++++
C. dolichotrichum Ames	2190	2/14/49	10	++++
C. elatum Kunze ex Fr.	1868	9/28/42	17	+++
C. erectum Skolko & Groves	2192	3/9/49	10	+**
C. funicolum Cooke	1869	9/28/42	17	+++
C. globosum Kunze ex Fr.	1668	6/16/42	17	−
C. globosum Kunze ex Fr.	1669	12/7/45	14	−
C. globosum Kunze ex Fr.	1870	9/8/42	17	+
C. indicum Cda.	2174	1/5/49	10	+
C. indicum Cda.	2194	2/14/49	10	+++
C. olivaceum Cooke & Ellis	1294	3/7/55	4	+**
C. reflexum Skolko & Groves	2195	2/14/49	10	++++
Claviceps purpurea (Fr.) Tul.	1270	7/6/42	17	+++
Dactylomyces thermophilus Sopp	1563	10/15/46	13	++++
Dasyscypha nivea (Hedw. ex Fr.) Sacc.	1637	6/10/42	17	−
Dipodascus uninucleatus Biggs	1629	6/10/42	17	+
Gymnoascus sp.	1712	7/6/42	17	+++
Kernia brachytricha (Ames) Benj.	1298	7/13/42	17	++++
Lophotrichus martenii Benj.	1716	2/3/42	17	+++**
Microascus doguetii Moreau	1715	2/3/42	17	+
M. lunatus Barron	1571	2/3/42	17	+++**
M. trigonosporus Emmons & Dodge	1570	2/3/42	17	++++
M. sp.	1689	6/22/42	17	++++
Mollisia (?) sp.	1638	6/9/42	17	−
Monascus purpureus Went	1596	6/8/42	17	+++
M. ruber v. Tiegh.	1597	1/16/46	13	+**
M. sp.	1211	6/19/42	17	+++
M. sp.	1991	2/8/45	14	−
M. sp.	1753	4/17/42	17	+
Myxotrichum conjugatum Kuehn	1244	2/5/42	17	++++
M. thaxteri Kuehn	1714	7/6/42	17	++++
Neurospora crassa Shear & Dodge	2223	1/5/50	9	++++
N. tetrasperma Shear & Dodge	1690	6/16/42	17	++++**
N. tetrasperma Shear & Dodge	2164	7/26/48	11	++++
Paecilomyces marquandii (Mass.) Hughes	1691	6/25/42	17	++++
P. varioti Bainier	691	5/19/42	17	++++
P. varioti Bainier	1122	5/22/42	17	++++
P. varioti Bainier (*P. aureo-cinnamomeum* (Biourge) Thom)	1127	6/5/42	17	++++
P. varioti Bainier (*P. mandschuricum* (Saito) Thom)	1128	6/22/42	17	++++
P. varioti Bainier	1674	6/22/42	17	++++
P. sp.	1113	7/6/42	17	++++
P. sp.	1126	5/22/42	17	++++
P. sp.	1569	6/22/42	17	+**
Pseudoarchniotus roseus Kuehn	1677	5/3/45	14	++++
Sclerotinia convoluta Dray.	1647	6/10/42	17	−
S. fructicola (Wint.) Rehm	1645	6/10/42	17	−
Sordaria curvicolla Wint.	2202	4/25/49	10	−
S. fimeti Wint.	1607	6/9/42	17	−
S. fimicola (Rob.) Ces. & de N.	1558	6/5/42	17	−
S. humana (Fckl.) Wint.	2198	2/14/49	10	++++
S. setosa Wint.	2201	6/30/49	10	+++

TABLE I—*Continued*

Name	NRRL no.	Date processed	Approx. age in years	Viability*
Thielavia sepedonium Emmons	1697	6/19/42	17	+
FUNGI IMPERFECTI				
Acremoniella sp.	1662	6/22/42	17	−
Acrothecium robustum Gilman & Abbott	1663	6/19/42	17	−
Alternaria circinans (Berk. & Curt.) Bolle (*A. oleracea* Milb.)	1680	6/16/42	17	++++
A. circinans (Berk. & Curt.) Bolle (*A. oleracea* Milb.)	2167	2/2/49	10	++++
A. solani (Ell. & G. Martin) Sor.	1806	3/30/42	17	−
A. tenuis Nees	2169	1/5/49	10	++++
A. sp.	1864	9/28/42	17	−
A. sp.	1683	6/22/42	17	+
Aspergillus candidus Lk. ex Fr.	1602	6/9/42	17	++++
A. candidus Lk. ex Fr.	1610	6/1/42	17	++++
Blastotrichum pennarum	1562	6/22/42	17	+++
B. sp.	1579	6/10/42	17	−
Bonordeniella aspera Linder	1580	6/19/42	17	−
Botryosporium sp.	1710	6/19/42	17	++
Botrytis spectabilis Harz	1776	6/1/42	17	++++
B. sp.	1285	11/28/48	11	+**
B. sp.	1633	6/9/42	17	++++
B. sp.	1634	6/22/42	17	−
B. sp. (*cinerea*-type)	1648	6/10/42	17	−
B. sp.	1650	6/10/42	17	+++
B. sp.	1777	6/19/42	17	−
Brachysporium sp.	1881	10/2/42	17	++++
Catenularia fuliginea Saito	1191	5/19/42	17	+++
C. sp.	1299	6/4/45	14	+++
Cephalosporium sp.	2182	1/5/49	10	++++
Cephalotrichum medium (Sacc.) Hughes (*Stysanus medius* Sacc.)	1878	10/2/42	17	++++
C. nanum (Ehrenb.) Hughes	1587	6/8/42	17	++
C. stemonitis (Pers.) Link	1840	6/9/42	17	+++
C. (*Stysanus*) sp.	1586	6/10/42	17	++++
Cercospora rubi Sacc.	1601	6/10/42	17	+
Chloridium musae Stahel	1636	6/9/42	17	++++
Cladosarum olivaceum Yuill & Yuill	374	3/2/42	17	+
Cladosporium fulvum Cooke	1671	6/16/42	17	++++
C. herbarum (Pers.) Lk. ex S. F. Gray	2175	1/5/49	10	++++
C. sp.	1643	6/10/42	17	+
C. sp.	1670	6/16/42	17	++++
C. sp.	1672	6/16/42	17	++++
Clonostachys sp.	1797	3/24/42	17	++
Colletotrichum lindemuthianum f. *alpha* (Sacc. & Magn.) Briosi & Cav.	1804	7/26/48	11	−
Curvularia brachyspora Boed.	2176	1/5/49	10	++++
C. falcata (Tehon) Boed.	2177	2/21/49	10	++++
C. lunata (Wakk.) Boed.	2178	1/5/49	10	++++
C. sp.	1679	6/19/42	17	++++
C. sp.	1692	6/16/42	17	++++
Didymocladium sinensis Thaxt.	1567	6/8/42	17	+++
Diplocladium sp.	1639	6/19/42	17	−
Fusarium bulbigenum Cke. & Mass. var. *lycopersici* (Brushi) Wr. & Reinking	1985	1/5/45	14	+++
F. lini Bolley	2204	4/20/49	10	++++**
F. moniliforme Sheldon	1675	12/7/45	14	+

TABLE I—*Continued*

Name	NRRL no.	Date processed	Approx. age in years	Viability*
Fusarium oxysporum Schlecht.	1871	9/28/42	17	−
F. sp.	1581	6/10/42	17	+++**
F. sp.	1603	6/9/42	17	++++
F. sp.	1605	6/9/42	17	+++
F. sp.	1699	6/26/42	17	+**
F. sp.	1866	9/28/42	17	+++
F. sp.	1943	11/30/43	16	+++
Gliocladium catenulatum Gilman & Abbott	1079	6/22/42	17	+++
G. deliquescens Sopp	1086	5/22/42	17	++++
G. fimbriatum Gilman & Abbott	1089	8/24/42	17	++++
G. fimbriatum Gilman & Abbott	1090	5/22/42	17	+++
G. penicilloides Cda.	1082	6/5/42	17	+++
G. roseum (Lk.) Thom	1084	5/22/42	17	+
G. vermoeseni (Biourge) Thom	1752	4/15/42	17	++++
G. sp.	1769	5/6/42	17	++
Gliomastix convoluta (Marchal) Mason	2179	1/5/49	10	++++
Graphium sp.	1582	6/19/42	17	++++
G. sp.	1872	9/28/42	17	+++
Haplographium chlorocephalum (Fres.) Grove	2208	5/25/49	10	++++
Humicola brevis (Gilman & Abbott) Gilman	1682	6/22/42	17	−
H. grisea Traaen	2180	2/2/49	10	+++
H. sp.	1879	10/2/42	17	++
Memnoniella echinata (Riv.) Gall.	1694	6/16/42	17	+++
M. echinata (Riv.) Gall.	1982	9/13/44	15	++
Metarrhizium anisopliae (Metsch.) Sor.	1945	12/1/43	16	++
M. sp. (No. "1" strain)	1944	12/1/43	16	+++
Monilia brunnea Gilman & Abbott	1686	6/19/42	17	++++
M. sitophila (Mont.) Sacc.	1275	5/7/42	17	++++
Myrothecium convexum Berk. & Curt.	1684	6/19/42	17	+++
M. roridum Tode	2183	1/5/49	10	++++
M. verrucaria (Alb. & Schw.) Ditm.	2184	1/5/49	10	+++
M. verrucaria (Alb. & Schw.) Ditm.	1874	9/28/42	17	++
M. verrucaria (Alb. & Schw.) Ditm.	2003	5/23/45	14	++++
M. verrucaria (Alb. & Schw.) Ditm.	1800	3/27/42	17	++++
Periconia synospora Fres.	2185	2/14/49	10	+++
Pestalotia sp.	2203	3/9/49	10	++++
Phialophora americana (Nannf.) Hughes (*Cadophora americana* Nannf.)	1631	6/9/42	17	++++
P. repens (Davidson) Conant	1632	6/9/42	17	++
P. richardsiae (Nannf.) Conant	1630	6/4/42	17	++++
Pullularia pullulans (Dby.) Berkhout	1673	6/16/42	17	++++
Rhinotrichum niveum Cooke & Mass.	1585	6/8/42	17	−
Scopulariopsis brevicaulis (Sacc.) Bainier	1096	6/4/42	17	+++
S. brevicaulis (Sacc.) Bainier	1101	5/22/42	17	+++
S. brevicaulis (Sacc.) Bainier var. *alba* Thom	1106	6/5/42	17	+++
S. brevicaulis (Sacc.) Bainier var. *glabra* Thom	1109	6/4/42	17	++++
S. brevicaulis (Sacc.) Bainier var. *hominis* Brumpt & Langeron	1110	6/4/42	17	++++
S. casei Loub.	1111	6/5/42	17	++++
S. constantini Bainier	1860	7/16/42	17	+++
S. sp.	1848	7/13/42	17	+++
S. sp.	1849	7/13/42	17	+++
S. sp.	1846	7/13/42	17	+++
S. sp.	1873	9/28/42	17	+++
Sepedonium xylogenum Sacc.	1576	6/25/42	17	−

TABLE I—*Continued*

Name	NRRL no.	Date processed	Approx. age in years	Viability*
S. sp.	1611	6/1/42	17	+++
S. sp.	1619	6/9/42	17	+++
Spadicoides xylogenum (A.L.Sm.) Hughes	1693	6/16/42	17	−
Sporobolomyces sp.	1584	6/8/42	17	−
Stachybotrys chartarum (Ehrenb.) Hughes (*S. atra* Cda.)	1877	9/28/42	17	+
S. lobulata Berk.	1695	6/16/42	17	++++
S. papyrogena Sacc.	1884	10/2/42	17	+++
Stemphylium asperulum Sacc.	1620	6/4/42	17	+
S. consortiale (Thuem.) Groves & Skolko	2187	1/5/49	10	++++
S. sarciniforme (Cav.) Wilts.	2188	2/2/49	10	++
S. solani Weber	1805	3/30/42	17	+
S. sp.	1621	6/4/42	17	+++
S. sp.	1883	10/2/42	17	++++
Streptothrix roseopurpureus	1578	6/8/42	17	++++
Thielaviopsis paradoxa (De Seyn.) Hoehn.	1803	3/27/42	17	++++
Trichoderma koningi Oud.	1761	4/17/42	17	++++
T. lignorum (Tode) Harz	1762	4/17/42	17	++++
T. viride (Pers.) Harz	1862	12/8/47	12	++++
T. sp.	1163	6/2/42	17	++++
T. sp.	1181	6/3/42	17	++++
T. sp.	1195	6/3/42	17	++++
T. sp.	1698	6/16/42	17	++++
T. sp.	1700	6/16/42	17	++++
Trichophyton mentagrophytes (Robin) Blanch. (*gypseum*-type)	1942	11/30/43	16	++++
T. mentagrophytes (Robin) Blanch. (*interdigitale*-type)	1295	6/19/42	17	+++
T. mentagrophytes (Robin) Blanch. (*interdigitale*-type)	1941	11/30/43	16	++++**
Trichothecium roseum Lk. ex Fr.	1588	6/8/42	17	++++
T. roseum Lk. ex Fr.	1665	6/16/42	17	++++
T. roseum Lk. ex Fr.	1708	6/2/42	17	++
Tritirachium dependens Limber	1210	5/27/47	12	++++**
T. roseum van Beyma	2018	6/12/46	13	+
Verticillium albo-atrum Reinke & Berth.	1204	6/10/42	17	++++
V. sp.	1847	7/13/42	17	++++
ACTINOMYCETES				
Micromonospora sp.	B-943	3/7/49	10	++++
Nocardia asteroides (Epp.) Blanch.	B-970	8/23/49	10	+++
Streptomyces fradiae (Waks. & Curt.) Waks. & Henr.	B-131	11/30/48	11	++
S. fradiae (Waks. & Curt.) Waks. & Henr.	B-556	12/14/48	11	++++
S. griseolus (Waks.) Waks. & Henr.	B-1062	2/9/50	9	+++
S. griseus (Krainsky) Waks. & Henr.	B-148	10/29/46	13	++++
S. griseus (Krainsky) Waks. & Henr.	B-599	3/15/49	10	++++
S. rubrireticuli Waks. & Henr.	B-946	3/21/49	10	+++
S. venezuelae Ehrlich et al.	B-902	11/30/48	11	++++
S. violaceus (Rossi-Doria emend. Krass.) Waks.	B-122	12/5/49	10	++++
S. sp.	B-590	3/15/49	10	+++
S. sp.	B-602	12/14/48	11	++
S. sp.	B-603	12/14/48	11	++++
S. sp.	B-604	12/29/48	11	++++
S. sp.	B-683	11/10/49	10	++++
S. sp.	B-684	3/15/49	10	++++
S. sp.	B-945	3/15/49	10	++++

Cultures derived from the lyophilized tubes were examined macroscopically and microscopically and were then compared, except for the Actinomycetes, with the plates started from the agar slants. The rating of the viability corresponds to that used by Raper and Alexander (**1945**), Fennell et al. (**1950**) and Mehrotra and Hesseltine (**1958**). When no growth resulted from the material in the first lyophil tube, further tubes of the strain were opened until growth occurred or the supply of lyophil tubes was exhausted. In case more than one tube had to be opened, the

TABLE II

CULTURES THAT WERE NOT VIABLE FROM LYOPHILIZATION

Spores present in tubes	NRRL no.	Spores not present in tubes	NRRL no.
MUCORALES		MUCORALES	
Mortierella sp.	1617	*Zygorhynchus moelleri*	1499
ENTOMOPHTHORALES		ASCOMYCETES	
Conidiobolus sp.	1612	*Chaetomium globosum*	1669
Delacroixia coronata	1912	*C. globosum*	1668
Entomophthora apiculata	1626	*Dasyscypha nivea*	1637
ASCOMYCETES		*Mollisia?* sp.	1638
Monascus sp.	1991	*Sordaria curvicolla*	2202
Sclerotinia convoluta	1647	*S. fimeti*	1607
S. fructicola	1645	*S. fimicola*	1558
FUNGI IMPERFECTI		FUNGI IMPERFECTI	
Botrytis sp. (*cinerea*-type)	1648	*Acremoniella* sp.	1662
B. sp.	1634	*Acrothecium robustrum*	1663
B. sp.	1777	*Alternaria solani*	1806
Diplocladium sp.	1639	*A.* sp.	1864
Humicola brevis	1682	*Blastotrichum* sp.	1579
Rhinotrichum niveum	1585	*Bonordeniella aspera*	1580
Sepedonium xylogenum	1576	*Colletotrichum lindemuthianum* f. *alpha*	1804
Sporobolomyces sp.	1584	*Fusarium oxysporum*	1871
		Spadicoides xylogenum	1693

pellet from the lyophil tube was placed directly on an agar plate. This concentration of material made it much easier to tell whether there were spores present or absent in the lyophil pellet.

RESULTS

Viabilities of the lyophilized strains are tabulated in TABLE I. Of the 363 cultures tested, 77.4% had excellent or good viability and 32 (8.8%) were not viable, most of the latter being Fungi Imperfecti. Seventeen (53%) of those not viable did not have spores in the lyophil tubes. Since we know that fungus cultures without spores never survive lyophilization by our process, these 17 strains should not be considered.

TABLE III

VIABILITY OF MUCORALES PRESERVED FOR 11 YEARS ON AGAR SLANTS UNDER MINERAL OIL AND FOR OVER 9 YEARS ON AGAR SLANTS IN AIR

Name	Agar slants covered with mineral oil			Malt agar slants		
	Total no.	No. pos.	No. neg.	Total no.	No. pos.	No. neg.
Absidia blakesleeana	7	1	6	7	7	0
A. butleri				3	0	3
A. capillata	1	1	0	1	1	0
A. coerulea	7	3 (2)*	4	7	0	7
A. corymbifera	1	0	1	1	1	0
A. cylindrospora	7	0	7	8	0	8
A. glauca	7	1	6	7	0	7
A. ramosa	8	2 (1)	6	8	8	0
A. ramosa var. *zurcheri*	1	0	1	1	1	0
A. repens	3	0	3	4	0	4
A. spinosa				1	0	1
A. sp.	1	0	1	1	1	0
Actinomucor elegans	2	1	1	2	0	2
Blakeslea trispora				1	0	1
Chaetocladium brefeldii				2	0	2
Circinella minor				1	0	1
C. simplex				1	0	1
C. muscae	3	0	3	11	0	11
C. umbellata	2	0	2	4	0	4
Cunninghamella bertholletiae	1	0	1	4	0	4
C. blakesleeana	2	0	2	7	0	7
C. echinulata	8	1	7	8	1	7
C. elegans	3	0	3	9	2	7
Helicostylum piriforme	4	2 (1)	2	4	0	4
Mortierella sp.	1	1	0	1	0	1
Mucor caninus	2	2	0	2	0	2
M. circinelloides	1	0	1	1	1	0
M. dispersus				2	0	2
M. genevensis	6	5 (1)	1	6	0	6
M. griseo-cyanus	9	8	1	9	2	7
M. hiemalis	1	0	1	1	0	1
M. lamprosporus	1	1	0			
M. microsporus	1	0	1	1	1	0
M. plumbeus	3	3	0	3	1	2
M. pusillus				1	0	1
M. racemosus	3	2	1	3	1	2
M. rouxii	1	0	1	2	0	2
M. silvaticus	1	0	1	1	0	1
M. sphaerosporus	1	1	0	1	0	1
M. tenius	1	1	0	1	0	1
M. III	4	4 (1)	0	4	2	2
M. IV	10	9 (1)	1	10	0	10
M. VII	2	2	0	2	0	2
"Red" *Mucor*	2	0	2	2	1	1
Mucor sp. "A"	1	1	0	1	0	1
M. sp. "B"	1	1	0	1	0	1
M. sp.	4	2	2	4	1	3
Parasitella parasitica	1	0	1	3	0	3

* Numbers in parentheses indicate the number of strains which had to be transferred more than once to get growth, hence the strain was almost dead.

TABLE III—*Continued*

Name	Agar slants covered with mineral oil			Malt agar slants		
	Total no.	No. pos.	No. neg.	Total no.	No. pos.	No. neg.
Phycomyces blakesleeanus	2	0	2	3	1	2
P. nitens	2	1 (1)	1	2	1	1
Rhizopus arrhizus	2	0	2	3	0	3
R. chinensis	1	0	1	1	0	1
R. cohnii	1	0	1	1	0	1
R. delemar	1	0	1	1	0	1
R. formosaensis	1	0	1	1	1	0
R. liquefaciens	2	0	2	2	1	1
R. microsporus	1	0	1	1	0	1
R. nodosus	2	1 (1)	1	2	0	2
R. oryzae	8	3 (1)	5	8	0	8
R. Peka I	1	0	1	1	0	1
R. reflexus	2	0	2	3	0	3
R. shanghaiensis				1	0	1
R. stolonifer	12	2 (1)	10	10	0	10
R. tritici	2	1	1	3	0	3
R. sp.	14	3 (2)	11	14	0	14
Syncephalastrum racemosum	1	0	1	1	1	0
S. sp.	7	4 (1)	3	7	7	0
Zygorhynchus exponens	1	0	1			
Z. heterogamus	3	3	0	3	1	2
Z. moelleri	8	6 (1)	2	8	0	8

Thus, the number of strains with spores which failed to survive lyophilization is 4.1% (TABLE II).

In all cases, the main morphological characteristics were retained by lyophilized cultures as well as, and sometimes with better than, those in cultures maintained by periodic transfer. For example, *Cephalotrichum stemonitis* (Pers.) Link NRRL 1840 had lost its ability to form synnemata by regular transfer but had retained it through lyophilization.

In April 1946, Dr. K. B. Raper, who was then in charge of the ARS Culture Collection, took a number of type Mucorales cultures from the collection and grew them on agar slants until maximum growth had been obtained. They were then covered with sterile mineral oil, stored in a cool room (5–6° C) and left undisturbed until June 1957, and then immediately transferred (large quantities of mycelium and spores were used) to Petri dishes of synthetic mucor agar, Hesseltine (**1954**). The cultures were incubated at 25° C for 10 days.

Viability tests of these cultures are tabulated in TABLE III. Out of 199 cultures tested, 79 were viable. In this instance only a negative or positive result was recorded. The reason for the low viability may be due to the fact that some of the tubes were not prepared adequately and

dried out on the surface of the slant, or the organisms may have used the oil. However, of those not covered with oil, several were still positive.

In September 1945, slants were made of a number of Mucorales strains on malt agar, Haynes et al. (**1955**). The slants gradually dried out at 5–6° C. In January 1955, they were flooded with a layer of malt agar. If the cultures were viable, they would grow on the fresh medium. The viability of these slants are also recorded in TABLE III. Of the 241 cultures tested, 196 (81.4%) were not viable. Again, only a negative or positive result was recorded.

DISCUSSION

The data presented show that lyophilization is clearly a superior method of preservation for most fungi. More than half of the few exceptions were fungi that had no spores when lyophilized. Spores or mycelial structures functioning as spores must be present before lyophilization, especially in the Fungi Imperfecti. The results and conclusions are about the same as those of Raper and Fennell (**1945**) and Fennell et al. (**1950**). But the time which one can safely expect most fungus species to survive in lyophilization is extended by our data. Viability of five of the negative or very poor strains had been negative or very poor in 1945. These are *Conidiobolus* sp. NRRL 1612, *Entomophthora apiculata* NRRL 1626, *Botrytis* sp. NRRL 1285, *Chaetomium globosum* NRRL 1669 and *Sordaria fimicola* NRRL 1558. As a whole, the only group we found that had adequate spores but that failed to survive lyophilization are members of the Entomophthorales. Morphology remained constant and was even better preserved in some instances in lyophil than by periodic transfer on agar slants. The mineral oil overlay method was not satisfactory with the strain of Mucorales we tested, since less than 50% were viable. Agar slants are of little value for preservation of fungus cultures when compared to lyophilization.

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SUMMARY

1. Representative lyophil cultures of the Mucorales, Entomophthorales, Ascomycetes, Fungi Imperfecti and Actinomycetes, the majority being 17 years old, were opened. Of the 363 cultures tested, 331 were viable. Seventeen of those not viable did not contain spores.

2. Morphological characteristics remained constant during lyophilization and, in a few exceptions where differences occurred, they were better than those periodically transferred.

3. Of the 199 cultures of Mucorales preserved by the oil overlay method for more than 11 years, 79 were viable.

4. Of the 241 cultures of Mucorales preserved on malt agar slants for more than 9 years, 45 were viable.

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COCHLIOBOLUS INTERMEDIUS, THE PERFECT STAGE OF CURVULARIA INTERMEDIA [1]

R. R. NELSON [2]

The genus *Curvularia* was established by Boedijn (1) in 1933 to include a group of phragmosporous Dematiaceae in which the most distinguishing character is the disproportinate size of the middle cell or cells of the conidium. Boedijn established three groups of species based on the septation and curvature of the conidia. The "maculans" group had 3-septate spores in which the 2 middle cells are disproportionately large. Originally, Boedijn transferred 13 species from other genera and described 5 new species, one of which was *Curvularia intermedia* Boed. *C. intermedia* is distinguished from other species in the "maculans" group in that the conidia are mostly slightly curved, whereas conidia of other species are usually straight.

Hitherto, no perfect stage has been associated with any species of *Curvularia*. As a part of a continuing study of the evolution of sexuality in fungi, the perfect stage of *C. intermedia* was obtained in paired cultures of conidial isolates. This paper reports the methods used, discusses the morphology and taxonomy, and presents evidence of heterothallism.

METHODS AND MATERIALS

Four conidial cultures and 130 monoascosporic isolates were used. Three of the conidial cultures were isolated from soil and the fourth from dead corn leaves.

Mature perithecia were obtained when mycelium of compatible isolates was placed on opposite sides of a section of sterile corn leaf placed in the center of a Petri plate containing Sachs' nutrient agar. Fertile perithecia developed most abundantly and consistently when paired cultures were grown on a medium of pH 4 and kept continually at 24° C. Most of the functional asci contained mature spores after 24 days.

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MORPHOLOGY AND TAXONOMY

Mature perithecia are black and ellipsoidal to globose, measuring 370–580 μ in height and 340–560 μ in diameter. A well-defined ostiolate beak, subconical to paraboloid and 150–280 μ long, is usually produced. A mass of hyaline cells frequently covers its apex. The locule is filled with a mass of hyaline, filamentous pseudoparaphyses. Asci are produced among the pseudoparaphyses and arise from the base of the locule. The asci are cylindrical to clavate, straight or slightly curved, measure 130–250 × 14–25 μ (mean 195 × 18.8 μ) and have short stipes. Asci are unitunicate and contain 1–8 ascospores coiled in a close helix. The ascospores are discharged by the splitting of the upper part of the ascus wall. The ascospores are filiform to flagelliform and somewhat tapered at the extremities. Mature ascospores are typically hyaline, have 5–9 septa, and measure 165–330 × 5–10 μ (mean 260 × 6.1 μ). Germination is terminal or lateral.

The fungus belongs in the genus *Cochliobolus,* erected by Drechsler (2) to include species formerly considered in *Ophiobolus,* but differing in having filiform ascospores in helicoid arrangement. Considering *Curvularia intermedia* to be a distinctive conidial stage and because no perfect stage has hitherto been associated with *C. intermedia,* the fungus is described as a new species of *Cochliobolus,* as follows:

Cochliobolus intermedius Nelson, sp. nov.

Ascomata nigra, ellipsoideo-globosa, ostiolata, 370–580 μ altitudine 340–560 μ diametro; rostrum ostiolare definitum, subconicum, usque ad paraboloideum, 150–280 μ longitudine, ad apicem saepe a massa cellulari hyalina tectum; loculus massa pseudoparaphysium hyalinarum filamentosarum et ascorum impletus; asci cylindrico-clavati, recti vel subcurvati breviter stipitati, apparenter unitunicati, 130–250 × 14–25 μ (mediani 195 × 18.8 μ); parte superiore fissente et ascosporas ejiciente; ascosporae 1–8, in helice stricta circinati, filiformes vel flagelliformes, ad apices attenuatae, hyalinae, 5–9-septatae, 165–330 × 5–10 μ (260 × 6.1 μ), tegumento tenui mucoso circumdatae, terminaliter vel lateraliter germinantes.

Specimens (*R. R. Nelson* No. 12118, TYPE, cultured on leaves of *Zea mays,* 1/61) have been deposited in the National Fungus Collections, Beltsville, Maryland, and in the Herbarium of the Royal Botanic Garden, Kew.

Heterothallism: Studies with selfed and paired cultures of conidial and ascosporic isolates of *C. intermedius* have proved that the species is heterothallic. Mature perithecia were produced only when monoascosporic or monoconidial isolates were grown in pairs.

Mature perithecia were obtained initially in a mating of conidial iso-

lates C12 and C118. In subsequent matings, isolates C16 and C45 were cross-fertile with C12. The conidial isolates were put into 1 of 2 compatibility groups, designated as *A* (isolate C12) and *a* (isolates C118, C16, and C45). One hundred and thirty monoascosporic isolates obtained from the 3 conidial matings were backcrossed to the parental isolates; 61 belonged to group *A* and 69 to group *a*. The segregation of isolates obtained from any specific cross never deviated appreciably from a 1:1 ratio, indicating that compatibility in *C. intermedius* is controlled by a single major gene locus. Variability in degree of compatibility was observed when ascosporic isolates were mated to parental isolates.

DISCUSSION

When Drechsler established the genus *Cochliobolus*, all species transferred from *Ophiobolus* were associated with species of *Helminthosporium* that exhibited bipolar germination. Drechsler believed that all species of *Cochliobolus* would have conidial stages in the bipolar series of *Helminthosporium*. However, the finding of a *Trichometasphaeria* perfect stage of a bipolar species, *Helminthosporium turcicum* Pass., by Luttrell (**3**) and the association of a species of *Curvularia* with *Cochliobolus* in the present studies illustrate that there is no consistent association of *Cochliobolus* and bipolar species of *Helminthosporium*.

The fact that a variety of conidial stages have their perfect stages in one Ascomycete genus clearly illustrates a lack of "natural" taxonomic criteria in the classification of the Fungi Imperfecti. Establishment of genera of the Fungi Imperfecti on the basis of easily observable differences in conidial morphology is merely a matter of convenience. In the opinion of the present author, *Curvularia* is a valid genus, based on the distinguishing character of the disproportionate middle cell of the conidium. It is logical to group species with such a character into a single genus rather than have one or more species in several other genera that are morphologically distinct from other members.

The results of the studies on segregation of ascospore isolates indicate that compatibility in *C. intermedius* is controlled by a single major gene locus. Thus, it appears that compatibility in this species is controlled in a manner similar to that reported for other heterothallic species of *Cochliobolus*, including *C. sativus* (Ito & Kurib.) Drechs. (**7**), *C. heterostrophus* Drechs. (**4**), and *C. carbonum* Nelson (**6**). Differences in backcross fertility of ascospore isolates indicate that other genes condition the gene locus for compatibility. Such variability in degree of compatibility is probably controlled quantitatively in a manner similar to

that reported for *C. heterostrophus* (**5**) and observed in other species of *Cochliobolus*.

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GERMINATION RESPONSES OF USTILAGO TRITICI TELIOSPORES IN RELATION TO LYOPHILIZATION. II. EFFECTS OF THE GERMINATIVE MEDIUM ON SURVIVAL [1]

SHIRL O. GRAHAM [2]

Differences in the germinative responses of the smuts are discussed by Fischer and Holton (**2**). They point out the variable reactions of many species to the substrates on which they have been sown. Subjecting teliospores of *Ustilago tritici* (Pers.) Rostr. to a saturated atmosphere for 24 hr in post-lyophile treatments often increased the percentages of germination (**5**).

Thus, the germinability of lyophilized teliospores appears to be influenced by mechanisms involved in water uptake as well as by physical and physiologic forces imposed upon them by the substrate. In this case, mechanical and physiologic interactions are complex because of the state of the lyophilized spore.

Such interactions have been discussed in connection with the eutectics of indigenous salt solutions within the subliming cell (**6**). Denaturing of protein owing to salt concentrations during the process has been demonstrated in nonliving systems, but the method of lyophilization alleviated this. Species that survive these suggested eutectic transitions in sublimation may undergo similar thresholds of high salt concentrations again on hydration.

The complex interactions of indigenous salts in lyophilized spores and the natural salts in the germination medium may be further affected, at least during short periods, by the viscosity or pH of the germinative medium. The higher the viscosity of a medium the slower is the rate of ingress of water into the sublimed spore. This lengthens any critical transition-period of high salt concentrations. In contrast, increased viscosity of the germinative medium might be counteracting on over-all survival, since a highly viscous medium would tend to prevent too rapid

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water uptake, and hence prevent lysis or related protoplasmic disorganization.

Such unmeasured interactions suggest the possibility of misinterpretation of results obtained with spores preserved via lyophilization. This study has been aimed at elucidating the effects certain of the above factors may have on the germinability of sublimed teliospores on various media.

TABLE I

GERMINATION ON DIVERSE MEDIA OF LYOPHILIZED U. TRITICI TELIOSPORES STORED UNDER VACUUM AT 0° C FOR ONE YEAR

Medium (2% agar)	Mean per cent germination (18 hr)	pH of medium after germination
Neopeptone	23	6.6
Peptone colloid	34	7.3
Physiologic salt (0.85% NaCl)	36	6.2
Richard's solution (No carbohydrate)	37	6.1
Potato malt	41	6.4
Malt extract	44	5.4
Yeast extract	45	6.2
Richard's solution (Casein hydrolysate for KNO_3)	46	5.2
Water	48	6.4
Alfalfa meal	52	6.8
Corn meal	52	6.5
Prune	54	6.2
Richard's solution	56	5.6
V-8 juice	56	4.3
PDA-Difco	59	5.4
Nutrient (No NaCl)	59	5.4
Molasses (8%)	61	5.1
Bran infusion	61	6.6
Wheat straw infusion	63	6.9
PDA-fresh (w/peel)	65	6.0
Oatmeal infusion	65	6.4
PDA-fresh (no peel)	68	6.1
Horse dung infusion	69	7.0
Pea (fresh frozen) infusion	70	6.6
Lima bean (dry) infusion	73	7.2
Wheat germ	73	5.8
12% sucrose	74	6.3

SCREENING OF CULTURAL MEDIA.—Initially, 27 diverse media were screened, using lyophilized teliospores of *Ustilago tritici* which had been sealed under vacuum and stored for a year at 0° C. Natural and synthetic media of both organic and inorganic composition were evaluated. Colloids, carbohydrates, nitrogen sources, growth factors, and electrolytes normally present in cultural media were incorporated.

The media were prepared according to procedures given by the manufacturer (1), by Riker and Riker (8), or by Rawlins (7), except that the agar content of all media was standardized at 2%. Only de-

ionized, distilled water and reagent-grade chemicals were used. Any further modifications are indicated.

The 27 agars prepared were: 1) water, 2) potato dextrose (commercial Difco), 3) potato dextrose (freshly prepared without peels), 4) potato dextrose (freshly prepared including peels), 5) potato malt (commercial Difco), 6) malt extract, 7) yeast extract, 8) 8% molasses, 9) 12% sucrose, 10) nutrient (without NaCl), 11) peptone colloid, 12) neopeptone, 13) physiologic salt (0.85% NaCl), 14) Richard's solution, 15) Richard's solution (modified—no carbohydrate source), 16) Richard's solution (modified—0.1% Difco acid hydrolysate of casein for KNO_3), 17) horse dung infusion, 18) prune infusion, 19) V-8 juice, 20) alfalfa meal infusion, 21) corn meal infusion, 22) oatmeal infusion, 23) wheat bran infusion, 24) wheat straw infusion, 25) wheat germ infusion, 26) lima bean (dried) infusion, and 27) pea (fresh frozen) infusion.

In this evaluation and all subsequent studies, the following standard procedures were used. Teliospores of *U. tritici*, which had been lyophilized at 150 μ Hg for 2 hr, sealed under vacuum and stored in the dark at 0° C for over a year, were dusted onto 3 plates of each medium. The seeded plates were incubated at 22° C for 18 hr. To standardize the time, each plate was then subjected to ethyl acetate fumes to kill spores. Germination counts were made on 1000 spores (3 counts of 100 spores in different locations from 2 plates, and 4 counts from the third plate). The mean was determined as the per cent germination on a given medium. Lastly, the pH of each medium was measured after the germination counts.

The results (TABLE I) emphasize the erratic germination that occurs on different substrates with teliospores of smuts (**2**). Germination on water agar seems to be the logical base from which to evaluate the effects of the various constituents in the media tested. It is definite that organic nitrogen and inorganic salts reduce and that carbohydrates enhance germinability. Levels of interaction and compensation between inorganic salt-carbohydrate and carbohydrate-organic nitrogen sources are evident. Superimposed upon these is pH as an untested independent variable.

The relatively poor germination of *U. tritici* in the presence of yeast and malt extracts was unexpected. These extracts contain or foster derivation of the Vitamin B complex moiety. Such a response is the reverse of the usual results on culturing smuts (summarized in Fischer and Holton, **2**). Members of the Vitamin B complex are necessary for most smuts grown on culture media. This observation, plus the similar

antithetic germination responses of *U. tritici* to nitrogenous compounds found necessary for growth of smuts (2), clearly differentiates germination optima from cultural needs.

Twelve per cent sucrose agar induced the highest germinability. This medium was included because of its high osmotic concentration. It was hypothesized that:

1. There might be less lysis, as was the case in pollen germination studies (3).
2. The significance of slower uptake of water by sublimed spores might be indicated.

Which of these factors or whether the carbohydrate *per se* was critical was not clear. Consequently, studies were conducted to evaluate sucrose and salt sources, and sucrose-salt source interactions.

RESPONSES TO SUCROSE AND SOME INORGANIC SALTS.—Germinative responses to sucrose as a carbohydrate source were compared against the material in a concentration gradient as an independent rehydration factor. A series of media was prepared using an additive module of 2% sucrose concentrations. A range from 2 to 20% was evaluated.

Concurrently, NaCl in 2% agar at different concentrations was evaluated against a similar series containing 12% sucrose as well. In addition, KH_2PO_4 and NaH_2PO_4 commonly employed as buffering salts, and KCl (to contrast potassium and sodium ions) were investigated at different concentrations in combination with 12% sucrose agars. Standard procedures were employed.

It is obvious (TABLE II) that the responses to sucrose were nutritive in nature and not due to osmotic concentration effects. Further, lower percentages of the carbohydrate were as effective as higher percentages.

As expected, the higher the salt concentration in a medium, the lower the per cent germination. It is surprising that the potassium ion was more suppressive than the sodium ion and that the phosphate ion was more suppressive than the chloride ion, unless pH was a factor in the latter comparison. In any event, the phosphates so commonly used to buffer growth media reduced the germinability. The results reemphasize that a substrate best for growth is not necessarily the best for germination.

RESPONSES TO *p*H.—In evaluating pH it was assumed that germinative levels would be low because of the salts employed to produce and maintain hydrogen ion concentrations. However, if pH itself has an effect, the use of a standard molar concentration of a given buffer system should point this out. Hence, the 0.1 M citric acid–0.2 M disodium

TABLE II

THE GERMINATION OF YEAR-OLD LYOPHILIZED U. TRITICI TELIOSPORES STORED AT 0° C UNDER VACUUM IN RESPONSE TO SUCROSE AND SALT CONCENTRATIONS

Medium (2% agar)	Mean per cent germination (18 hr)	pH of medium after germination
Water	42	6.5
Sucrose		
2%	62	5.9
4%	66	5.9
6%	66	6.2
8%	65	6.4
10%	64	6.2
12%	66	6.4
14%	68	6.4
16%	65	6.4
18%	63	6.4
20%	63	6.4
Sodium Chloride		
1%	33	6.2
2%	29	6.2
4%	5	5.9
6%	trace	6.0
8%	0	5.8
10%	0	5.9
Sucrose (12%) plus: Sodium Chloride		
1%	47	6.2
2%	39	5.8
4%	16	5.6
6%	9	5.5
8%	0	5.6
10%	0	5.6
Potassium Chloride		
1%	39	5.4
2%	28	5.4
4%	13	5.5
6%	5	5.4
8%	2	5.4
Potassium Phosphate (Monobasic)		
1%	31	4.6
2%	17	4.5
4%	6	4.5
6%	1	4.5
8%	trace	4.5
Sodium Phosphate (Monobasic)		
1%	29	4.5
2%	19	4.4
4%	11	4.5
6%	4	4.5
8%	trace	4.5

phosphate system was employed from pH 3 to pH 8 (**4**). In addition, 0.1 M citric acid was used alone for a hydrogen ion concentration of about pH 2.2. Levels above pH 8 were not tested. To boost responses, 12% sucrose was incorporated into the agars.

The results (TABLE III) clearly indicate that pH is an independent variable in germination of teliospores. Its influence is ordinarily masked by interactions of other factors. The optimal pH for *U. tritici* apparently lies between pH 5 and pH 6.

TABLE III

THE GERMINATION OF LYOPHILIZED U. TRITICI TELIOSPORES IN RELATION TO HYDROGEN ION CONCENTRATION[a] OF 12% SUCROSE AGAR

pH of medium	Per cent germination
pH 2.2	0
pH 3	0
pH 4	27
pH 5	42
pH 6	47
pH 7	34
pH 8	20

[a] 0.1 M citric acid–0.2 M Na_2HPO_4 buffer system employed.

SUMMARY

The studies reported here point out the independent but important role of the germination substrate on the potential germinability of lyophilized teliospores. Various constituents, natural or additive to a medium, depress or enhance per cent germination. Organic nitrogen and salts in concentrations normal to culture media depressed germination; carbohydrates, particularly sucrose, enhanced germination. Considerable interaction between moieties was apparent. Hydrogen ion concentration was found to be an independent, though usually masked, contributing variable. As an accessory factor, pH appears to be optimal between pH 5 and pH 6. Germination was depressed linearly from this optimal range.

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COMPATIBILITY OF SEVERAL CLONAL LINES OF ERYSIPHE CICHORACEARUM [1, 2]

RALPH M. MORRISON

INTRODUCTION

Reports of the heterothallic nature of *Erysiphe cichoraceaarum* DC. ex Merat have been made by Yarwood (**1935**), Schnathorst (**1959**) and Morrison (**1961**). From this work it is evident that two mating types are involved. However, when these studies were expanded to include clonal lines of *E. cichoraceaarum* isolated from several different host plants, additional factors appeared to affect compatibility in certain lines. The present study is an attempt to resolve the identity of these compatibility factors.

METHODS

The method of culturing *Erysiphe cichoraceaarum* has been described by Morrison (**1961**). Leaf disk tissue was obtained from *Helianthus annuus, Xanthium pennsylvanicum* and *Zinnia elegans.* A total of forty-four clonal lines were isolated from *H. annuus* (13 clones), *X. pennsylvanicum* (8 clones), and *Z. elegans* (23 clones). Once the cultures produced enough conidiospores, transfers were made to uninfected leaf disk tissue. Where possible, all three species of host tissues served as substrate for the mildew clones. Each cross was done in quadruplicate. Distilled water, and 0.4 ppm kinetin in distilled water were used to sustain the leaf tissues. The culture chambers with the infected disks were incubated at 18° C at an illumination intensity of 300 ft-c. The photoperiod consisted of sixteen hours of light and eight hours of dark.

RESULTS

Mating Reactions. When clones of *Erysiphe cichoraceaarum* isolated from *Helianthus annuus* were mated with clones from *Zinnia elegans,*

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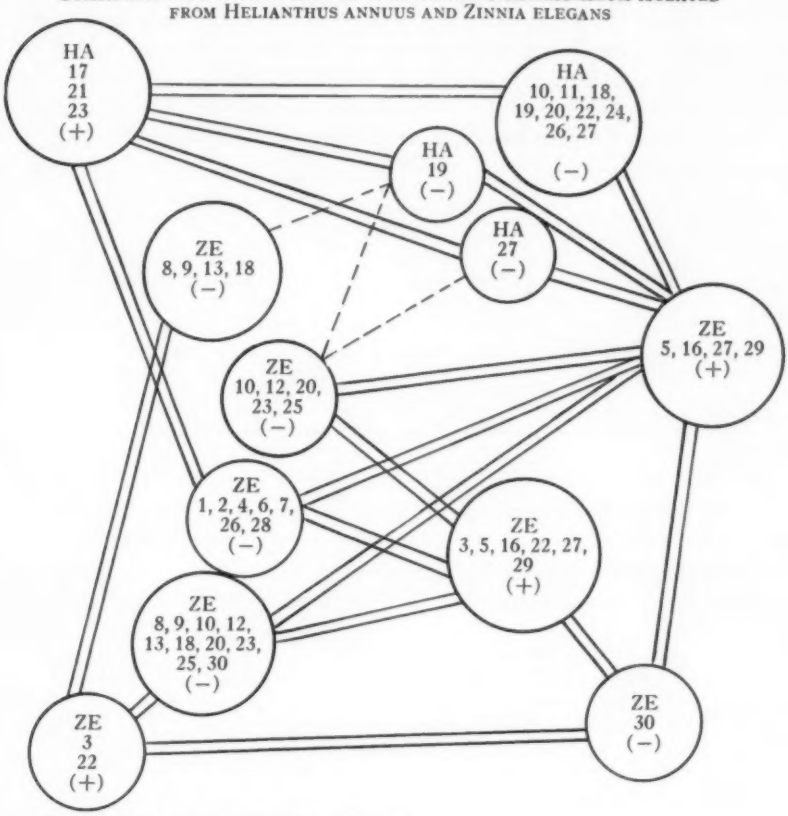
[2] The author wishes to express his appreciation to Dr. R. M. Johns for his valuable help in the preparation of this paper, and to Dr. V. M. Cutter, Jr., for his very helpful criticisms of this work.

TABLE I

COMPATIBILITY BETWEEN CLONES OF ERYSIPHE CICHORACEARUM ISOLATED FROM HELIANTHUS ANNUUS AND ZINNIA ELEGANS



Solid double lines = expected combinations.
Broken lines = unexpected combinations.
Clones HA $_{17}$, HA $_{21}$ and HA $_{23}$ were assigned mating type +; compatibility or noncompatibility with these clones was the basis for the mating type assignment to the remaining clones.

several different compatibility classes were observed (TABLE I). *Helianthus annuus* clones HA 10, 11, 18, 19, 20, 22, 24, 25, 26, and 27 were compatible with *Zinnia elegans* clones ZE, 5, 16, 27, and 29. *Helianthus annuus* clones HA 17, 21, and 23 were fertile with *Zinnia elegans* clones ZE 1, 4, 6, 7, and 26. *Helianthus annuus* clone HA 21 was crossed several times to ZE 2 and ZE 28 without ever forming cleistothecia. Clone HA 17 proved incompatible with ZE 28. *Helianthus annuus*

clones HA 19 and 27 were compatible with clones ZE 10, 12, 20, 23, and 25. Clones ZE 8, 9, 13, and 18 were compatible with clone HA 19 only. Clones ZE 3, 22, and 30 belonged to a completely incompatible group, as these clones did not produce any cleistothecia when mated to

TABLE II

CLONAL CROSSES OF ERYSIPHE CICHORACEARUM ISOLATED FROM XANTHIUM PENNSYLVANICUM

XP	7	9	1	3	4	5	2	6
7	−	−	−	−	−	−	+	+
9	−	−	−	−	−	−	+	+
2	+	+	+	−	−	+	−	−
6	+	+	−	−	−	−	−	−
1	−	−	−	−	−	−	+	−
3	−	−	−	−	−	−	−	−
4	−	−	−	−	−	−	−	−
5	−	−	−	−	−	−	+	−

Clones isolated from *Xanthium pennsylvanicum* in the greenhouse.

the clones of *E. cichoracearum* isolated from *H. annuus*. These crosses were performed several times confirming these data (TABLE I).

The clones of *Erysiphe cichoracearum* isolated from *Xanthium pennsylvanicum* were crossed in all possible combinations. The data from these crosses revealed that clone XP 2 was compatible with clones XP 1,

TABLE III

CLONAL CROSSES OF ERYSIPHE CICHORACEARUM ISOLATED FROM HELIANTHUS ANNUUS AND XANTHIUM PENNSYLVANICUM

XP	HA 17	21	23	18	19	20	26	27	28
2	−	−	−	−	+	−	−	−	−
4	−	−	−	+	+	−	−	−	−
6	−	−	−	−	+	−	−	−	−
7	−	−	−	−	−	−	−	−	−
1	−	−	−	+	−	−	−	−	−
3	−	−	−	−	−	−	−	−	−
5	−	−	−	−	−	−	−	−	−
9	−	−	−	−	−	−	−	−	−

5, 7, and 9. Clone XP 6 was compatible with clones XP 7 and 9. Clones XP 3 and 4 proved incompatible when mated (TABLE II). Clones of *E. cichoracearum* isolated from *Helianthus annuus* were then crossed to the *X. pennsylvanicum clones*. Clone HA 19 was compatible with clones XP 2, 4, and 6, while clone HA 18 was compatible with clones XP 1 and 4 (TABLE III). Abortive cleistothecia were pro-

duced by the following crosses: XP 4 × HA 23, XP 7 × HA 23, XP 2 × HA 26, XP 7 × HA 26, and XP 9 × HA 26. These cleistothecia never matured nor produced asci.

The cleistothecia which were produced as the result of compatible matings were formed on tissues supported by tap and distilled water and by the 0.4 ppm kinetin concentration. These structures were morphologically indistinguishable from those collected from intact plants in nature. Ten per cent of the cleistothecia (excluding the abortive cleis-

TABLE IV

THE RESULTS OF CROSS INOCULATIONS OF CLONAL STRAINS OF ERYSIPHE CICHORACEARUM ISOLATED FROM HELIANTHUS ANNUUS ONTO A VARIETY OF DIFFERENT HOST PLANTS

HA	*H. annuus*		*H. petiolaris*		*H. tuberosus*		*Z. elegans*		*X. pennsylvanicum*	
	D. W.	K	D. W.	K	D. W.	K	D. W.	K	D. W.	K
1*	+	+	+	+	+	+	−	−		
2*	+	+	+	+	+	+	−	−		
4*	+	+	+	+	+	+	−	−		
9*	+	+	+	+	+	+	−	−		
10	+	+	+	+	+	+	−	−	−	−
11	+	+	+	+	+	+	−	−	−	−
17	+	+	+	+	+	+	+	+	+	+
18	+	+	+	+	+	+	+	+	+	+
19	+	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	+	+
21	+	+	+	+	+	+	+	+	+	+
22	+	+	+	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+	+	+	+
24	+	+	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+	+	+
26	+	+	+	+	+	+	+	+	+	+
27	+	+	+	+	+	+	+	+	+	+

* Clones HA 1, 2, 4, and 9 were lost before the *X. pennsylvanicum* inoculations could be carried out.

+ = normal growth. − = no growth.

D.W. = distilled water.

K. = kinetin; 0.4 ppm for *Z. elegans* and *H. annuus* and 0.8 for *X. pennsylvanicum*.

tothecia found in several of the XP × HA crosses) produced in culture on the three species of host plants were either without asci, or when asci were present, they did not contain any ascospores after subsequent physical treatment. Four per cent of the cleistothecia of *Erysiphe cichoracearum* collected from *Helianthus annuus* found in the botany experimental field at Indiana University, Bloomington, lacked asci and ascospores. Eight per cent of the cleistothecia from *H. annuus* in the greenhouse were without asci and ascospores. Fourteen out of the

thirty-four nonascal cleistothecia were found to be infected with *Cicinnobolus cesatii* de Bary. Emmons (1930) reported that *C. cesatii* inhibited the formation of cleistothecia by *Erysiphe* spp. In many instances where cleistothecia were formed, *C. cesatii* converted them into pycnidia. When *C. cesatti* was found in a clonal cross, these were discarded and new crosses were made.

TABLE V

THE RESULTS OF CROSS INOCULATIONS OF CLONAL STRAINS OF ERYSIPHE CICHORACEARUM ISOLATED FROM ZINNIA ELEGANS ONTO A VARIETY OF HOST PLANTS

ZE	*H. annuus*		*X. pennsylvanicum*		*Z. elegans*	
	D.W.	K.	D.W.	K.	D.W.	K.
1	+	+	−	−	+	+
2	+	+	RGM	RGM	+	+
3	+	+	−	−	+	+
4	+	+	−	−	+	+
5	+	+	RGC	RGC	+	+
6	+	+	−	−	+	+
7	+	+	+	+	+	+
8	+	+	−	−	+	+
9	+	+	−	−	+	+
10	+	+	−	−	+	+
12	+	+	−	−	+	+
13	+	+	−	−	+	+
16	+	+	−	−	+	+
18	+	+	+	+	+	+
20	+	+	+	+	+	+
22	+	+	RGC	RGC	+	+
23	+	+	RGC	RGC	+	+
25	+	+	RGM	RGM	+	+
26	+	+	−	−	+	+
27	+	+	−	−	+	+
28	+	+	−	−	+	+
29	+	+	−	−	+	+
30	+	+	+	+	+	+

+ = normal growth. − = no growth. RGM = restricted growth mycelium. RGC = restricted growth conidial.

Cross Inoculation Experiments. During the course of clonal isolation and crossing, a number of cross inoculations were performed. That is, clonal isolates of *Erysiphe cichoracearum* harvested from one specific host, were inoculated on leaf tissue from other host species. Each clonal inoculation on a specific host tissue was done in quadruplicate. The culture conditions were the same as used for the mating experiments.

Helianthus annuus clones were inoculated onto leaf disk tissue of *H. annuus* L., *Helianthus petiolaris* Nutt., *Helianthus tuberosus* L.,

Zinnia elegans Jacq. and *Xanthium pennsylvanicum* Wallr. (TABLE IV). From these data it was found that all the clones of *Erysiphe cichoracearum* isolated from *H. annuus* infected *H. annuus, H. petiolaris* and *H. tuberosus.* Six clones isolated from *H. annuus,* HA 1, 2, 4, 9, 10, and 11 did not infect the *Z. elegans* leaf disks. Clones HA 10 and 11 did not infect the *X. pennsylvanicum* tissue.

Clones of *Erysiphe cichoracearum* isolated from *Zinnia elegans* were inoculated onto leaf disks of *Helianthus annuus, Xanthium pennsylvanicum* and *Zinnia elegans* (TABLE V). All the *Z. elegans* clones infected the *H. annuus* and *Z. elegans* leaf tissues. However, only

TABLE VI

THE RESULTS OF CROSS INOCULATIONS OF CLONAL STRAINS OF ERYSIPHE CICHORACEARUM ISOLATED FROM XANTHIUM PENNSYLVANICUM ONTO A VARIETY OF HOST PLANTS

XP	*H. annuus*		*X. pennsylvanicum*		*Z. elegans*	
	D.W.	K.	D.W.	K.	D.W.	K.
1	+	+	+	+	+	+
2	+	+	+	+	−	−
3	+	+	+	+	−	−
4	+	+	+	+	−	−
5	+	+	+	+	RGM	RGM
6	+	+	+	+	RGM	RGM
7	+	+	+	+	+	+
8	+	+	+	+	RGC	RGC
9*	+	+	+	+		
10*	+	+	+	+		

* Clones XP_9 and XP_{10} were lost before the *Z. elegans* tissue was inoculated.
+ = normal growth. − = no growth.
RGM = restricted growth mycelium. RGC = restricted growth conidial.

clones ZE 7, 18, 20, and 30 infected and grew normally on the *X. pennsylvanicum* leaf disk tissues. Clones ZE 2, 5, 22, 23, and 25 infected the *X. pennsylvanicum* tissues but subsequent growth of these clones was restricted. The other clones isolated from *Z. elegans* did not attack the *X. pennsylvanicum* tissues.

Erysiphe cichoracearum clones, isolated from *Xanthium pennsylvanicum,* were isolated onto leaf disk tissues of *Helianthus annuus, Xanthium pennsylvanicum* and *Zinnia elegans* (TABLE VI). All the *X. pennsylvanicum* clones of *E. cichoracearum* grew normally on *H. annuus* and *X. pennsylvanicum* tissues in culture. Clones XP 5, 6, and 8 infected the *Z. elegans* tissues, but subsequent development was limited. The other *X. pennsylvanicum* clones did not infect *Z. elegans* disks.

DISCUSSION

When clones of *Erysiphe cichoracearum* isolated from *Helianthus annuus* were crossed with clones isolated from *Zinnia elegans*, several different patterns of compatibility arose. Thirteen of the clones isolated from *H. annuus* were fertile with nine of the *Z. elegans* clones (Morrison, **1961**). The picture becomes more complex with the emergence of the pattern shown by the two clones from *H. annuus* which crossed with nine clones of *Z. elegans* to form cleistothecia. None of these cleistothecia produced ascospores, though they appeared morphologically normal. Both of these clones, presuming a two mating type system, and from data collected from the crosses between clones of *H. annuus* and *Z. elegans* (Morrison, **1961**), belonged to the same mating type class. When the clones of *Erysiphe cichoracearum*, isolated from *H. annuus* and *X. pennsylvanicum* were crossed, only four fertile matings emerged. However, in a number of crosses abortive cleistothecia were formed, these structures never developed past the mycelial knot stage in their growth.

These data reveal that along with the two suggested mating types, there are additional factors which determine the compatibility of clonal lines in *Erysiphe cichoracearum*. Hanna (**1925**) found that in some species of *Coprinus* Fr. the two sets of incompatibility factors may be alike in a species growing in the same locality, but one or both sets of incompatibility factors may be different in the same species growing in a different locality. In this case all sexual phases of one fungus would be compatible with all sexual phases of the other fungus. If one pair of factors was different in the two fungi, certain combinations would be compatible. Whitehouse (**1949**) explained this in terms of multiple allelomorphs, that is, when either of the incompatibility factors produced an allele by mutation, a cross became possible between fungi of the same species from different geographic locations. Mounce and Macrae (**1936**) found complete compatibility between collections made in different localities or on different hosts in *Lenzites saepiaria* (Wulf.) Fr. and in *Trametes americana* Overh. Barnett (**1937**) made similar observations for some of the Heterobasidae. Whitehouse (**1949**) pointed out the analogy between multiple allelomorphic heterothallism in the Hymenomycetes, and self-sterility in flowering plants. Mather (**1942**), in support of this analogy, pointed out that the incompatibility of pollen and style usually had as its genetic basis a multiple allelomorphic series at one locus. Mather has also shown that the second locus of the tetrapolar species can be regarded as an adaptation which promoted outbreeding by decreasing the frequency of fertile sister-matings. While multiple alleles

also favor outbreeding, they also can increase the frequency of fertile nonsister matings.

Although multiple allelomorphic heterothallism has not been established in the ascomycetes, interspecific hybridization and crosses between the same species from different geographic sources have confirmed the presence of at least two allelomorphs at the locus for heterothallism (Whitehouse, **1949**). It is proposed, therefore, that there are two allelomorphic series in *Erysiphe cichoracearum* which control the compatibility of single conidial clones isolated from different host plants and host specificity. Mutations of the compatibility factors could then conceivably produce allelomorphs which would permit certain "illegitimate" combinations involving, for example, a plus clone isolated from *Helianthus annuus* mating with a plus clone from *Zinnia elegans;* as well as inhibiting combinations between "legitimate" clones of different mating types from different hosts.

Cross Inoculation Experiments. From the data obtained in the cross inoculation experiments, it appeared that *Helianthus annuus* is a universal host for all the clones isolated from it, from *Xanthium pennsylvanicum* and from *Zinnia elegans.* Few clones isolated from *Z. elegans* grew well on the *X. pennsylvanicum* tissue, while only two clones isolated from *X. pennsylvanicum* developed normally on the *Z. elegans* tissue. These data suggest that clones of *Erysiphe cichoracearum* from different host plants have been isolated into specific physiological races. Salmon (**1904**) referred to such a process as the evolution of "biologic forms" or the process of adaptation to different host plant species by the fungal parasite. Moseman (**1956**) has obtained similar results with single ascospore lines of *Erysiphe graminis.*

SUMMARY

The crossing data confirm heterothallism in *E. cichoraccarum* and establish the presence of two compatibility factors in this organism. Many isolates from *Helianthus, Xanthium,* and *Zinnia* are fertile with one another. Other results, confirmed by repeated matings, suggest that there are two allelomorphic series in *E. cichoracearum* which control the compatibility of single conidial lines isolated from different host plants and the host specificity of these clones.

During the course of clonal isolation and crossing, a number of cross inoculations were performed. Clonal isolates taken from one host plant, inoculated on leaf tissue from another species of host plants, grew on the *Helianthus annuus* tissue. The *Zinnia elegans* clones showed

very little compatibility for the *Xanthium pennsylvanicum* tissue; the *X. pennsylvanicum* clones had little affinity for the *Z. elegans* tissue. These data suggest that clones of *Erysiphe cichoracearum* isolated from different host plants are being separated into specific physiological races.

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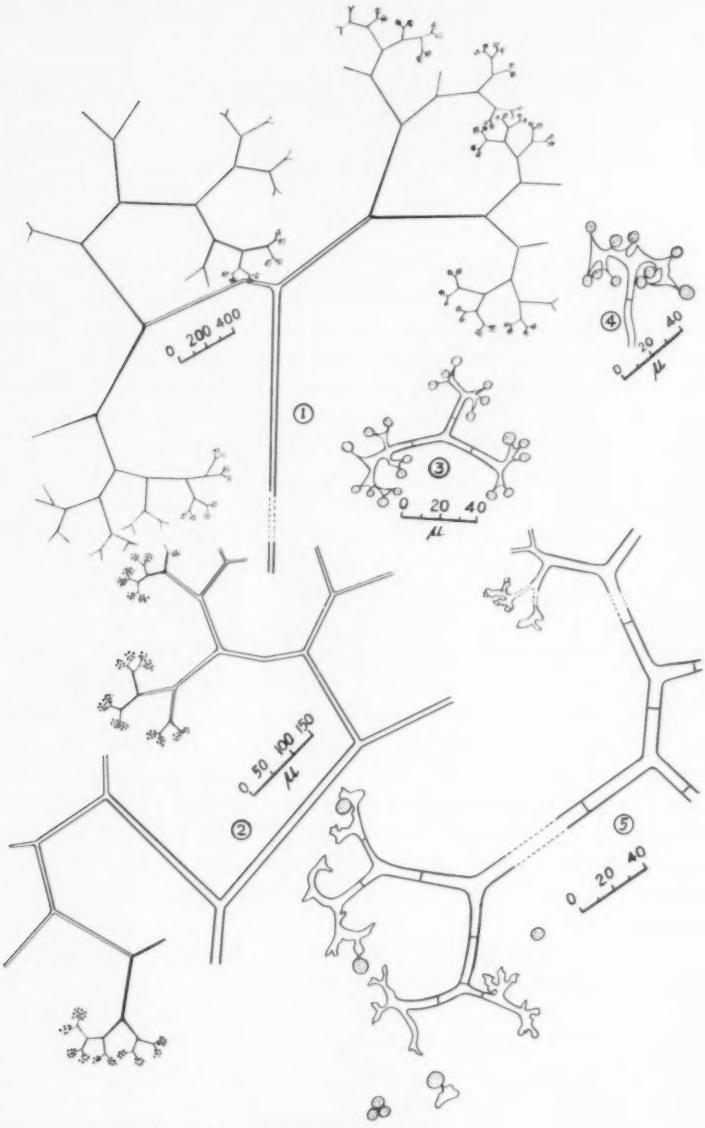
A NEW SPECIES OF CHAETOCLADIUM FROM INDIA

B. S. Mehrotra and A. K. Sarbhoy

(with 8 figures)

The genus *Chaetocladium* Fres. was placed by van Tieghem (**1875**) in his Tribe Mucorées; Schroeter (**1886**), Fischer (**1892**), Lendner (**1908**) and Fitzpatrick (**1930**) kept it in the family Chaetocladiaceae. Naumov (**1939**) assigned it a position in the Tribe Thamnidieae which he had created by merging the two families Thamnidiaceae and Chaetocladiaceae. Zycha (**1935**), on the other hand, placed *Chaetocladium* in the family Thamnidiaceae with which he had merged the family Chaetocladiaceae. Hesseltine and Anderson (**1957**) considered Zycha's treatment more nearly natural than those of the previous authors. The genus *Chaetocladium* is characterized firstly, by its repeatedly branched conidiophores, whorls of branches arising from the main conidiophore, the ultimate whorls being short and swollen distally with very thin short stalks (sterigmata) which bear the conidia; secondly, by the presence of sterile spines extending from the central axis of the whorl of ultimate branches; thirdly, by the presence of conidia (one-spored sporangiola) and the absence of many-spored sporangiola and sporangia. It is similar to *Chaetostylum* in its manner of branching of the conidiophores and in the presence of sterile spines, but differs from it in having exclusively conidia (one-spored sporangiola) and in the complete absence of sporangia. It resembles *Cunninghamella* in the production of conidia (one-spored sporangiola), but differs from it in the manner of branching of the conidiophores and in the presence of sterile spines (Hesseltine and Anderson, **1957**, p. 37).

The genus *Chaetocladium* has never been reported from India. Recently the authors came across an isolate from a soil sample of a cultivated field in Allahabad. This isolate resembled *Chaetocladium* in two of the essential characters enumerated above, viz., the branching pattern of the conidiophore and the presence of conidia (single-spored sporangiola) only, but it lacked sterile spines. In a recent treatment of the genus, Hesseltine and Anderson (**1957**) have recognized two species, *Chaetocladium jonesii* (Berk. & Br.) Fres. and *C. brefeldii* v. Tiegh. & Le Mon. The former species is recognized by its larger, rough-walled



CHAETOCLADIUM HESSELTINII SP. NOV.

FIGS. 1–5.

conidia which have been shown to be monosporangiola by van Tieghem and Le Monnier (**1873**, pl. 23, Fig. 65). On the other hand the latter species is characterized by smaller (3–7 μ) and nearly smooth-walled conidia. Our isolate is apparently a *Chaetocladium*, but differs from the two known species in the following characters:

(a) The absence of spines. The ovoid swellings at the ends of the ultimate branches do not extend as spines beyond the enlargements. Only in old conidiophores do the naked stalks (sterigmata) look like spines due to the dispersal of the conidia from their tips.

(b) The absence of growth at 7° C and poor growth at temperatures lower than 20°. The other two species grow at 7° C (Hesseltine and Anderson, **1957**, p. 41).

The species has been named after Dr. C. W. Hesseltine with whom the senior author has had the privilege of working and whose kind collaboration is a source of great help.

Chaetocladium hesseltinii Mehrotra & Sarbhoy, sp. nov. FIGS. 1–8

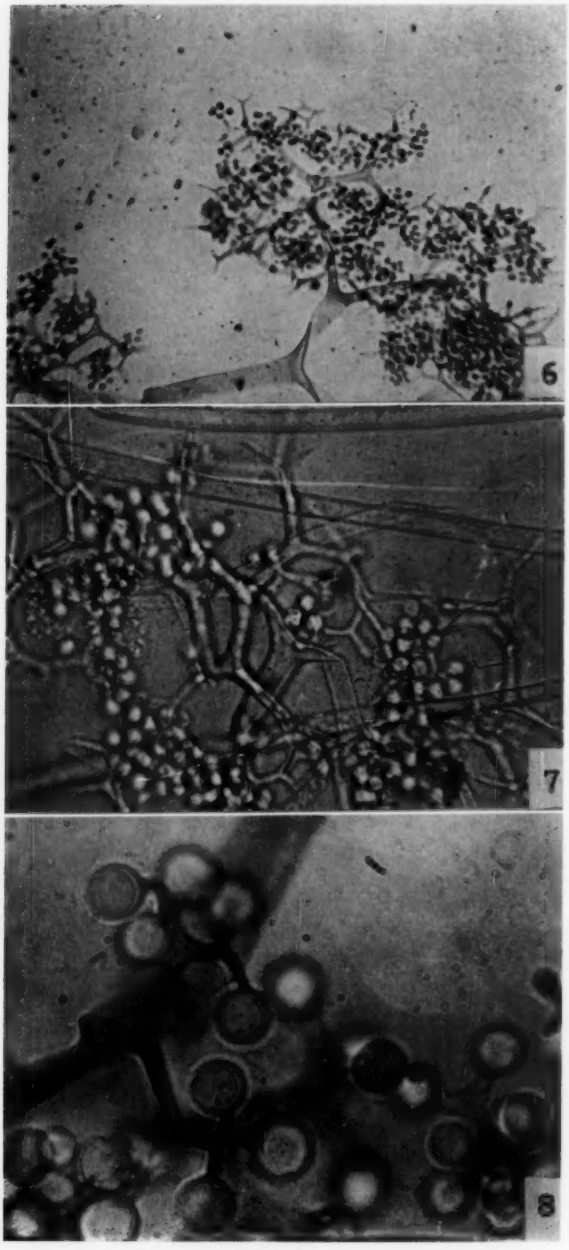
Coloniae cito crescentes supra SMA et PDA ad temp. 25°–35° C, operculum patinae (Petri-dish) tangentes, incremento crassissimo prope punctum inoculationis, primo incolorae, deinde plus minusve olivaceobubalinae, facie versa alba vel pallidissime lutea; stolones semper adsunt et monstrant evolutionem rhizoidearum dum substrato adjacent; conidiophori emergentes verticillati ex hyphis aereis, aliquoties dichotome divisi ramulis ultimis desinentibus in tumescentias ovoideas 7.5–12 μ diam., qui producunt stipites breves fastigatos conidia ferentes, ut plurimum 3.3 μ lati ad basin, 1.6 μ in regione apicali et 9.9–52.8 μ longi; conidia globosa vel ovata, hyalina, tenuiter pallide luteola, globosa quidem 4.8–6.6 μ, ovata vero 5–9.9 × 3.5–8.5 μ (medietate 5 × 3.5 μ); sporangia, multispora sporangiola et chlamydosporae absunt; zygosporae non visae. Species nova notata non est parasitice inficiens Mucorinas alias; gallarum formatio haud evidens.

Colonies growing fast on SMA [1] and PDA [2] at temperatures 25°–35° C, touching the lid of the Petri-dish, with heaviest growth near the point of inoculation, at first colourless but later near olive buff, in reverse white to very light yellow; stolons always present and showing rhizoidal development when in contact with substrate; conidiophores arising in whorls from aerial hyphae, several times dichotomously di-

[1] SMA: Dextrose 40 g, asparagine 2 g, potassium acid phosphate 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, thiamine chloride 0.5 mg, Agar-agar 2%, distilled water 1000 cc.

[2] PDA: Peeled potatoes 200 g, glucose 20 g, Agar-agar 2%, distilled water 1000 cc.

FIGS. 1–5. *Chaetocladium hesseltinii*. 1, 2. Conidiophore, showing branching patterns. 3, 4. Ultimate branches with ovoid swellings showing attachment of the conidia. 5. A mature conidiophore after most of the conidia have fallen.



FIGS. 6–8.

vided; ultimate branches ending in ovoid swellings 7.5–12 μ in diam, which give rise to short, tapering, conidia-bearing stalks; stalks generally 3.3 μ at the base and 1.6 μ wide in the apical region and 9.9–52.8 μ in length; conidia globose to oval, smooth, hyaline, slightly yellowish, globose 4.8–6.6 μ, oval 5–9.9 × 3.5–8.5 μ, average 5 × 3.5 μ; sporangia, multispored sporangiola and chlamydospores absent; zygospores not seen. Parasitism on other mucors not observed and no gall formation evident.

Isolated from soil of a cultivated field in Allahabad, pH 6.5. TYPE: Culture deposited at NURD, Peoria, Illinois, U.S.A., as NRRL No. 5912. Also, a culture deposited in the culture collection of the Botany Department, University of Allahabad as No. M-20. Dried specimens have been deposited at the herbarium of the Botany Department, University of Allahabad, India.

ACKNOWLEDGMENTS

The authors are grateful to Dr. C. W. Hesseltine for kindly giving his valued opinion on the isolate, and to Dr. H. Santapau for the Latin diagnosis. Thanks are also due to the Scientific Research Committee, U.P., India, for the contingency grant.

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FIGS. 6–8. *Chaetocladium hesseltinii*. 6, 7. Photomicrograph showing branching pattern of the conidiophores; 6 × 200, 7 × 600. 8. Photomicrograph of few ultimate branches of the conidiophore (note the absence of spines) × 1600.

SARCINOMYCES INKIN IN BRASIL

HECTOR AURICH MESONES AND CARROLL W. DODGE

(WITH 2 FIGURES)

Sarcinomyces Inkin Oho (1919) was first isolated and described from a scrotal lesion somewhat resembling eczema marginatum of Hebra in Formosa and the lesion was reproduced experimentally on a human volunteer by rubbing the organism into excoriations on the scrotum. A search of the literature has not revealed other cases; apparently the two cases reported here are the second and third cases known. Since *Sarcinomyces* infections respond to treatment similar to that used in eczema marginatum caused by *Epidermophyton floccosum* (Harz) Langeron & Milochevitch, perhaps cases have been diagnosed and treated by physicians without isolating the etiologic agent and hence other cases of *Sarcinomyces* infection have not been recognized.

CASE HISTORIES

Two medical students, ages 19 and 20 years, of Peruvian origin, consulted us for a mild but continuous pruritus of small lesions on the scrotal and inguinal regions. The lesions were circular, vesciular, about 2 cm in diameter, covered with thin, moist scales and crusts. Later smaller, secondary lesions were observed on the penis and in the interdigital spaces of the hands, apparently from autoinoculation. The lesions begin as a group of vesicles in the Malpighian layer, surrounded by an erythematous halo, spreading peripherally. After a few days, the lesions become scaling and crusted, probably from friction of the clothing. The temperature at this time of year was about 30° C and the relative humidity seldom dropped below 85%, producing a very moist skin most of the time. In the scrotal lesions, a few hyperkeratotic nodules were noted with some thickening of the papillary layer.

CULTURES

The fungus was easily isolated in pure culture on Sabouraud agar from the scales and crusts. The colonies were yeastlike, thin, pure white, slightly umbonate at the point of inoculation, only slightly shining with a somewhat pitted surface, more noticeable in culture tubes than

on Petri plates. The giant colony on plates in about two weeks (FIG. 1) is about 5 cm in diameter, thin, deeply lobed, with smooth rounded margins and low submarginal folds which do not crack on drying. On semisolid media, the folds are longer and resemble the veins of a fig leaf. Dextrose, sucrose, maltose, lactose and mannitol were assimilated as carbon sources, with only slight growth on urea.

FIG. 1. Giant colony of *Sarcinomyces Inkin* Oho on Sabouraud agar, about two weeks old. Natural size.

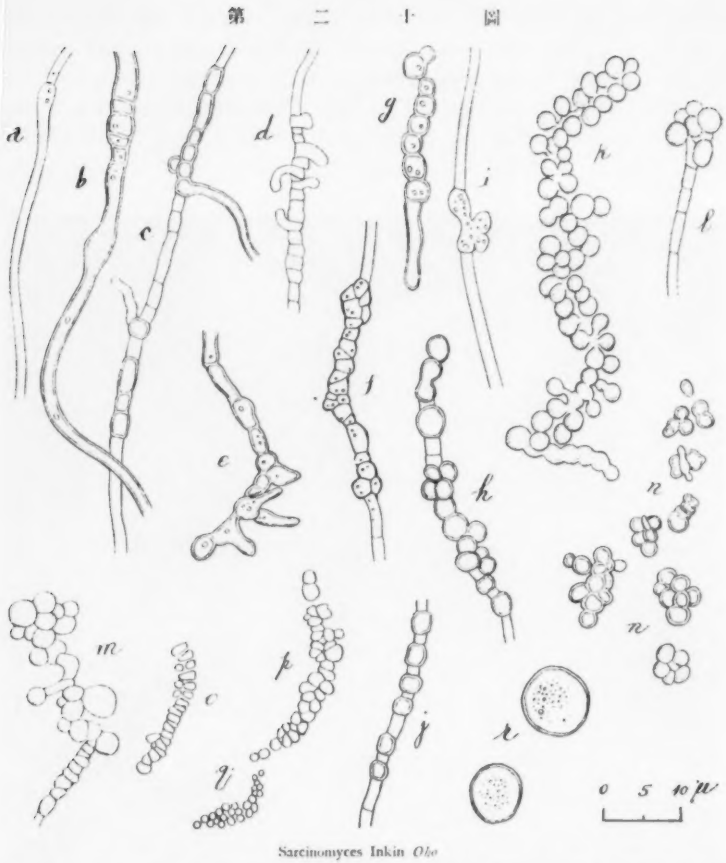

FIG. 2. *a*. portion of hypha, with one end of cell slightly swollen, either degenerate racket mycelium or an incipient chlamydospore; *b*, *c*, *e*, *g*, *h* and *j*. intercalary chlamydospores, either single or in chains; *d*. incipient branching; *f*. lower portion of *m*; *p*. Sarcina-like septation; *o*. Sarcina-like cells separating; *i*. initial of blastospore; *k*, *l*. upper portion of *m*, blastospore production; *n*. groups of small chlamydospores developed from blastospores; *r*. giant senescent cells (after Ota, 1926).

MORPHOLOGY

The mycelium is slender, irregularly septate, about 2 μ in diameter, with occasionally one end of a cell slightly swollen (FIG. 2, a) but we have been unable to decide whether it is degenerate racket mycelium or an incipient chlamydospore. Along some of the hyphae, intercalary

chlamydospores, either single or more often in chains, are found (*b*, *c*, *g*, *h*, *j*). Other hyphae show lateral, pyriform to subspherical blastospores up to about 4 μ in diameter (*k*) or in subterminal groups (*l*). Along other hyphae, the septa are very close, some oblique or even longitudinal (*f*, lower portion of *m*), the cells finally separating (*o* and *p*) and tending to round up (*q*). The interpretation of the cell groups (*n*) is less clear but they seem to be blastospores transforming into small chlamydospores, as they were seen only in preparations from old cultures beginning to dry out. FIG. 2, *d*, seems to be initials of branches, structures rarely seen in our cultures.

We have never been able to decide whether cell division is actually in three planes as figured for *Sarcinomyces crustaceus* and *S. albus*, upon which Lindner (**1898**) based the genus. The cell walls of *S. Inkin* are never rough as figured for *S. crustaceus*. The cultures were lost by an unfortunate laboratory accident before photomicrographs could be prepared and FIG. 2 illustrating the morphology is reproduced from Ota (**1926**) as we have been unable to locate a copy of Oho (**1919**), the original publication of *S. Inkin*. The same figure is also reproduced by Fonseca (**1943**), a work long out of print. We have repeatedly observed the morphology as figured, from cultures of both patients.

TREATMENT

After preliminary treatment with calamine lotion to relieve the pruritus while the fungus was being isolated and identified, andriodermol both powder and ointment, was used and the lesions healed in about a week with no recurrence. Andriodermol is a Brasilian proprietary preparation with an undecylenic acid base.

SUMMARY

Sarcinomyces Inkin was isolated several times from each of two cases in Brasil, observed in August 1959. The cultural characters and the morphology are described and figured with a note on treatment of the cases.

ACKNOWLEDGMENTS

We wish to express our gratitude to Professor Alvaro de Figueiredo, Cadeira de Parasitologia, Faculdade de Medicina, Universidade do Recife, while the senior author was Research Assistant and the junior author was Visiting Professor in the Cadeira, for his interest and pro-

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NOTES AND BRIEF ARTICLES

Fine Structure of Mycota

2. Demonstration of the Haustoria of Lichens[1]

In the course of a broad survey of fungi with the electron microscope species of two genera of lichens have been examined. These are *Cladonia cristatella* Tuck. and *Lecidea* sp. Both belong to the type genera in their respective families of the Lecanorales. The first is a macrolichen growing on soil and, by virtue of its bright red apothecia, one of the best known. The particular species of the second is diminutive and was represented by a few small colonies on rotting wood.[2]

It is the current consensus of opinion that when the mycobiont-type of haustoria occur they generally clasp the surface of the algal cell forming an appressorium and that more rarely do they penetrate through the cell wall of the phycobiont as far as the plasma membrane.[3] If, however, the evidence presented here is typical of the two genera, then the latter type of haustoria may not be as uncommon as previously supposed. (*Cladonia* and *Lecidea* are the two largest lichen genera and represent a total of 367 species—125 and 242 respectively.[3]) Figure 1 is of *Lecidea* sp. and shows the fungal wall (FW) penetrating the algal wall (AlW) and branching to form a bifurcate haustorium (H). Figure 2 shows a simple (in section) haustorium of *Cladonia cristatella*. (Haustoria similar to these may be the "small flattened peg-like outgrowths" that have been observed to form during the artificial synthesis of *Lecanora dispersa* (Pers.) Röhl.[4])—R. T. Moore[5] and J. H. McAlear,[5] Department of Plant Pathology, Cornell University and Division

[1] This work was supported in part by Grant H-3493 from The National Heart Institute, Public Health Service, and in part by a research fellowship, 9197-C1, to the senior author from the National Institute of Allergy and Infectious Diseases, Prof. R. P. Korf, sponsor.

[2] The authors wish to express their appreciation to Dr. M. E. Hale, Jr., for the identification of *Lecidea* sp.

[3] Hale, M. E., Jr. 1957. Lichen Handbook. Smithsonian Inst., Washington, D. C.

[4] Ahmadjian, V. 1959. A contribution toward lichen synthesis. Mycologia **51**: 56–60.

[5] Present address: Electron Microscope Laboratory, University of California, Berkeley.

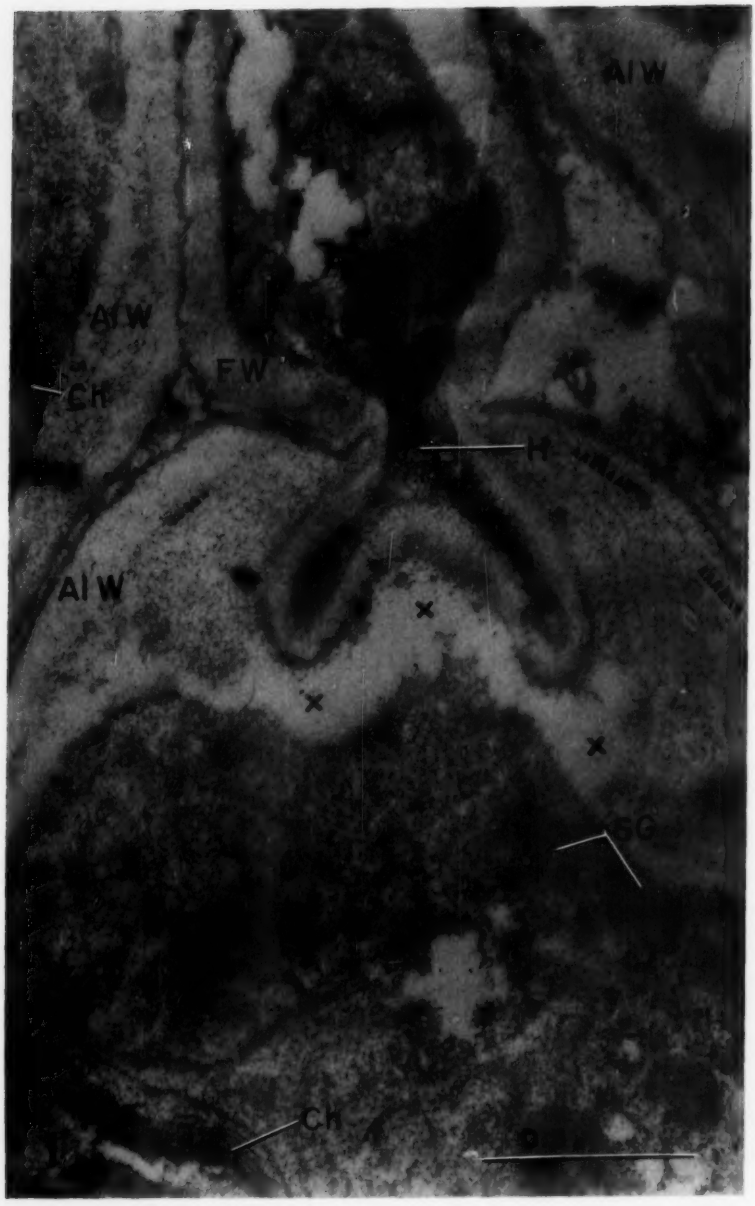

FIG. 1.

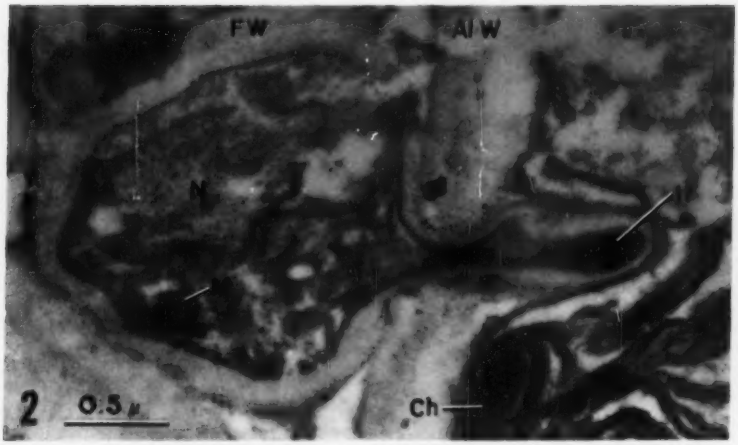

FIG. 2. *Cladonia cristatella*. AlW, algal wall; Ch, chloroplast; FW, fungal wall; N, M, nucleus and mitochondrion of the fungal cell; H, haustorium of the fungus. × 30,000.

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A NOTE ON VARIETIES OF PUCCINIA MENTHAE

The mint rust fungus, *Puccinia menthae* Pers., is a highly variable species consisting of several intergrading morphologic forms. In a previous study (Baxter, Lloydia **22**: 244. 1959), four varieties of *P. menthae* were described. This note presents the results of further studies of morphologic variation in this species. Specimens examined were obtained through the courtesy of Dr. G. B. Cummins, Purdue University and Dr. F. D. Kern, Pennsylvania State College. This study was supported by a grant from the National Science Foundation.

PUCCINIA MENTHAE Pers. var. **basiporula** Baxter, var. nov.

A forma typica speciei differt teliosporis 18–21 × 24–29 μ, poro cellulae inferiore in parte media sito.

TYPE SPECIMEN: On *Ocimum* sp. (probably *Ocimum micranthum* Willd.), slopes of Salto de Tequendama, Dept. Cundinamarca, Colombia, July 6, 1929, *Chardon* (Chardon Fungi of Colombia 643).

FIG. 1. *Lecidea* sp. AlW, algal wall (arrows indicate the juncture between the cytoplasm and the cell wall); Ch, chloroplast; SG, storage granules in the algal cell; FW, fungal wall; H, haustorium of the fungus; ×, space due to shrinkage during polymerization of the embedding plastic (methacrylate). × 60,000.

In *P. menthae* var. *basiporula* the pore in the lower cell of the teliospore is located midway between the septum and the pedicel. In all other respects this variety resembles collections of *P. menthae* from eastern North America and Europe.

The type material of this variety was examined during the course of a study of the rust fungi parasitizing species of *Hyptis*. The host plant, previously considered to be *Hyptis mutabilis spicata* (Poit.) Epl., is actually a species of *Ocimum*. I am indebted to Dr. Carl Epling, University of California, Los Angeles, for determination of the host material.

PUCCINIA MENTHAE Pers. var. **pseudomenthae** (G. H. Cunn.) Baxter, stat. nov.

Puccinia pseudomenthae G. H. Cunn., Trans. N. Z. Inst. **61**: 409. 1930.

A forma typica speciei differt teliosporis 17–24 × 22–30 μ, membrana verrucosa vel verrucosa-aculeata, poro cellulae inferiore in parte media vel juxta pedicellum sito.

TYPE SPECIMEN: On *Mentha cunninghamii* Benth., Franz Josef Glacier, Westland, New Zealand, *Allan and Cunningham*.

The distinguishing characteristics of this variety are the uniform, nonumbonate teliospore wall and the location of the pore in the lower cell of the teliospore. In size and wall characters the teliospores resemble those of a form of *P. menthae* occurring on *Pycnanthemum pilosum* Nutt. in the central United States.—JOHN W. BAXTER, University of Wisconsin, Milwaukee.

THE 1958 FORAY OF THE MYCOLOGICAL SOCIETY OF AMERICA

The 1958 Foray was held in Indiana centering around Bloomington, site of the meetings of the American Institute of Biological Sciences. The collecting trips were made on Aug. 22 and 23, the Friday and Saturday preceding the Annual Meeting of the Society. Housing was provided in dormitories on the Campus of Indiana University.

On Friday collections were made in McCormick's Creek State Park. Trails to be used for this purpose were selected by Dr. Cummins and the Park Naturalist. The morning and afternoon trips were taken in different parts of the Park so that a variety of habitats could be sampled with least disturbance to or from that part of the public which was also

using the Park. An excellent lunch was served in the restaurant in McCormick's Creek Inn.

Saturday's collections were made in two areas in Brown County State Park. Areas chosen were off the beaten path and provided habitats for a number of different kinds of fungi so that collectors in all groups were satisfied with the material obtained. Less collecting was done at Brown County because many of the species present there had been collected at McCormick's Creek.

Because the habitats are so similar in each of the two locations, the accompanying list is a compilation of the collecting done during the two days without regard to location. This list may be taken as indicative of the mycobiota of south central Indiana during the latter part of a summer which was dryer than usual but which was not suffering from drought.

A roster was signed at the dinner which was served to members and guests at the Abe Martin Lodge at Brown County State Park Saturday afternoon. On it were recorded 68 names including 14 inactive persons and 54 persons who were on the trails at some time during the Foray. Eighteen of these people turned in lists from which the accompanying master list has been compiled. It includes 426 species of saprobes, and plant pathogens, many of which were collected upon observing obvious fruit bodies, lesions on plants, etc., and some of which were collected by culturing samples of soils and other habitats upon return to individual laboratories. A list of collectors, preceded by a code letter, is presented. Specimens or cultures can be studied upon application to the collector whose code letter follows the name of the fungus in the master list.

Several interesting records were made as a result of this Foray. On the last afternoon in Brown County State Park, Dr. C. W. Emmons found in a young hickory tree a beautiful collection of *Septobasidium lilacinoalbum*. This record contributes to the growing list of species of this genus found north of the Ohio River, is probably the first record for the genus in Indiana, and justified the bringing to the Foray of Dr. Martin's machete. The third specimen known for *Hypochnella violacea* and the genus *Hypochnella* was found at McCormick's Creek; this will aid in a better understanding of this obscure genus. *Cerotelium tanake* was found in Brown County State Park on the ground nut, *Amphicarpa bracteata*. It was previously known only in Japan. *Rhynchosporina tridentis* on *Triodia flava* was previously known only in Kansas. A species of *Uromyces* on *Carex platyphylla* defies further identification at this stage in our knowledge of sedge rusts. *Poria latitans* was previously known to Dr. J. L. Lowe only from Florida, Mississippi and Arkansas. Collections of Discomycetes in these two Parks are con-

tributing to the knowledge of the systematics of these fungi in North America. Range extensions of other species listed here result from the fact that the fungus biota of Indiana has never been thoroughly investigated.

A—Maria Pantidou, Plant Research Institute, Central Experimental Farm, Ottawa.

B—C. W. Hesseltine, A.R.S. Culture Collection, Northern Utilization Research and Development Laboratories, Peoria.

C—Wm. Bridge Cooke, R. A. Taft Sanitary Engineering Center, Cincinnati.

D—Ross W. Davidson, Beltsville Forest Disease Laboratory, Beltsville.

E—Chester W. Emmons, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland.

F—Mr. and Mrs. Oswaldo Fidalgo, Sao Paulo, Brazil.

G—Morris Gordon, State Health Laboratory, Albany, New York.

H—Kenneth Harrison, Kentville, Nova Scotia.

J—G. T. Johnson, Department of Botany, University of Arkansas, Fayetteville.

K—Richard P. Korf and L. R. Batra, Department of Plant Pathology, Cornell University, Ithaca, New York.

L—Robena Luck-Allen and Roy F. Cain, Department of Botany, University of Toronto, Toronto.

M—G. W. Martin, Botany Department, State University of Iowa, Iowa City.

N—C. T. Rogerson, New York Botanical Garden, New York.

O—E. A. Atwell, Forest Products Laboratories, Ottawa.

P—G. B. Cummins, Department of Plant Pathology, Purdue University, Lafayette.

R—D. P. Rogers, Botany Department, University of Illinois, Urbana.

S—E. G. Simmons, Pioneering Research Division, Quartermaster Research and Engineering Command, Natick.

W—L. J. Wickerham, Northern Utilization Research and Development Labs., Peoria.

Z—W. G. Ziller, Forest Biology Laboratory, Victoria, British Columbia.

MYXOMYCETES: Ceratiomyxales, Ceratiomyxaceae: *Ceratiomyxa fruticulosa* (Muell.) Macbr.—M; Liceales: *Licea minima* Fr.—L; Reticulariaceae: *Lycogala epidendrum* (L.) Fr.—G, J, M; Cribrariaceae: *Dictydium cancellatum* (Batsch.) Macbr.—J; Trichiales, Trichiaceae: *Arcyria cinerea* (Bull.) Pers.—J, M; *A. denudata* (L.) Wettst.—J, M; *A. digitata* (Schw.) Rost.—J; *Hemitrichia clavata* (Pers.) Rost.

—J; *H. serpula* (Scop.) Rost.—G, J, M; *H. stipitata* (Mass.) Macbr. —G, M; *H. vesparium* (Batsch.) Macbr.—G, J, M; *Trichia favoginea* (Batsch.) Pers.—G, J, M; Stemonitales, Stemonitaceae: *Stemonitis ferruginea* Ehr.—J; *S. fusca* Roth—G, L, M; *S. splendens* Rost.—M; *S.? virginiensis* Rex—G; Physarales, Physaraceae: *Fuligo septica* (L.) Web.—J, M; *Physarella oblonga* (Berk. & Curt.) Morg.—M, J; *Physarum contextum* Pers.—M; *P. globuliferum* (Bull.) Pers.—J, M; *P. nutans* Pers.—M, J; *P. wingatense* Macbr.—G.

PHYCOMYCETES: Oomycetes, Chytridiales, Synchytriaceae: *Synchytrium decipiens* Farl. on *Apios tuberosa*—P; Zygomycetes, Mucoraceae: *Absidia glauca* Hagem—B; *A. orchidis* (Vuill.) Hagem—B; *Mucor fragilis* Bainier—B; *M. lamprosporus* Lendner—B; *M. oblongisporus* Naumov—B; *M. ramannianus* Moeller—B; *M. subtilissimus* Oudemans —B; *Zygorhynchus moelleri* Vuill.—B; *Syzygites megalocarpus* Ehrh. ex Fr.; Mortierellaceae: *Mortierella* sp.—B; *M. humilis* Linnemann— B; Choanephoraceae: *Choanephora trispora* (Thaxter) Sinha (−)—S; *C. circinans* (Neganishi & Kawakami) Hesseltine & Benjamin—B; *Cunninghamella elegans* Lendner—B; Entomophthorales, Entomophthoraceae: *Massopsora cicadina* Pk.—K.

ASCOMYCETES: Hemiascomycetes, Endomycetales, Saccharomycetaceae: *Hansenula anomala* (Hansen) H. & P. Sydow—W; *Saccharomyces delbrueckii* Lindner—W; *S. mangini* Guill.—W; Euascomycetes, Eurotiales, Eurotiaceae: *Carpenteles javanicum* (van Beyma) Shear— B; Elaphomycetaceae: *Elaphomyces* sp. (as host of *Cordyceps*)—L, S; Dothideales, Phyllachoraceae: *Phyllachora graminis* (Pers.) Fckl. on *Hystrix patula*—P, on *Panicum* sp.—P; *P. lespedezae* (Schw.) Sacc. on *Lespedeza* sp.—L; Hypocreales, Nectriaceae: *Nectria sanguinea* Fr. —L; Hypocreaceae: *Claviceps purpurea* (Fr.) Tul. on *Hystrix patula* —P; *Cordyceps ophioglossoides* (Ehrh.) Link on *Elaphomyces* sp.—L, S; *Hypocrea gelatinosa* (Tode ex Fr.) Fr.—L, N; *H. lactea* Fr.—N; *H. patella* Pk.—L, N; *H. rufa* (Pers. ex Fr.) Fr.—L, N; *H. schweinitzii* (Fr.) Sacc.—N; *H. sulphurea* (Schw.) Sacc.—N, O; *Hypomyces hyalinus* (Schw. ex Fr.) Tul. on *?Amanita* sp.—N; *H. lactifluorum* (Schw. ex Fr.) Tul. on *?Lactarius* sp.—N; *H. rosellus* (Alb. & Schw. ex Fr.) Tul.—N; Sphaeriales, Fimetariaceae: *Podospora anserina* (Ces.) Wint. —L; *P. setosa* (Wint.) Niessl—L; Sphaeriaceae: *Acanthostigma decastylum* (Cke.) Sacc.—L; *Bertia moriformis* (Tode ex Fr.) de Not.— L, N; *Coniochaeta ligniaria* (Lév.) Mass.—L; *Lasiosphaeria hirsuta* (Fr.) Ces. & de Not.—L; *L. ovina* (Pers. ex Fr.) Ces. & de Not.—L, N; *L. strigosa* (Alb. & Schw.) Sacc.—L; Gnomoniaceae: *Glomerella cingulata* (Ston.) Spauld. & Schrenck. (conidial)—L; *Gnomonia ulmea*

(Schw.) Thuem. on *Ulmus americana*—P; Valsaceae: *Eutypella scoparia* (Schw.) Ell. & Ev.—L; *E. stellulata* (Fr.) Sacc.—L; Diatrypaceae: *Diatrype* sp.—F; *D. stigma* (Hoffm.) de Not.—L; Melogrammataceae: *Endothia gyrosa* (Schw.) Fr. on *Fagus grandifolia*—N; Xylariaceae: *Daldinia concentrica* Ces. & de Not.—C, F, J, O; *Hypoxylon atropunctatum* (Schw. ex Fr.) Cke.—H, L; *H. cohaerens* Pers. ex Fr.—L; *H. fuscopurpureum* (Schw.) Berk.—L; *H. fuscum* Pers. ex Fr.—O; *H. perforatum* (Schw.) Sacc.—O; *H. rubiginosum* (Pers. ex Fr.) Fr.—L, O; *H. serpens* (Pers.) Fr.—L; *Rosellinia subiculata* (Schw.) Sacc.—L; *Ustulina vulgaris* Tul.—O; *Xylaria corniformis* Fr.—S; *X. castorea* Berk.—L; *X. hypoxylon* (Lév. ex Fr.) Grev.—C, L, O; *X. longipes* Nits.—L; *X. polymorpha* (Pers.) Grev.—C, D, F, J, O; Hysteriales, Hysteriaceae: *Glonium stellatum* Muhl. ex Fr.—H; Phacidiales, Phacidiaceae: *Coccomyces hiemalis* Higgins on *Prunus virginiana*—P.

Discomycetes: Helotiales, Helotiaceae: *Arachnopeziza delicatula* Fckl. —K; *A.* sp.—K; *Belonidium* sp.—K; *Calycella* sp.—K; *Catinella nigro-olivacea* (Schw.) Durand—K; *Chlorociboria rugipes* (Berk.) Raman & Korf in Raman, Korf & Batra—K; *Chlorosplenium chlora* (Schw.) Curt. in Sprague—K; *Dasyscyphus tricolor*—K; *D.* sp.—K; *Helotium* sp.—K; *H. fraternum* Pk.—K, *H. fructigenum* (Bull. ex Fr.) Karst.—K; *H. scutula* (Pers.) Karst.—K; *Hyalinia crystallina* (Quél.) Boud.—K; *H.* sp.—K; *H. rubella* (Pers. ex Fr.) Nannf.—K; *Hyaloscypha* sp.—K; *H. stevensonii* (Berk. & Br.) Nannf.—K; *Neorehmia ceratophora* Hoehn.—L; *Orbilia* spp.—K; *O. crenatomarginata* Hoehn. —L; *O. cyathea* Vel.—L; *O. luteorubella* (Nyl.) Karst.—L; *O. paradoxa* Vel.—L; Mollisiaceae: *Mollisia* sp.—K; *M. cinerea* (Batsch.) Karst.—L; Sclerotiniaceae: *Rutstroemia longipes* (Pk.) White—K; *R. macrospora* (Pk.) Kanouse in Wehmeyer—F, K, L; Geoglossaceae: *Cudonia* sp.—K; *Leotia lubrica* Pers.—F, K; *Microglossum olivaceum* (Pers. ex Fr.) Gill.—K; *Trichoglossum* spp.—K. Lecanorales: *Karschia* sp.—K. Pezizales, Pezizaceae: *Galiella rufa* (Schw.) Nannf. & Korf in Korf—K; *Helvella subclavipes* (Phill. & Ell.) Kobayasi—K; *H.* spp.—K; *Humaria hemisphaerica* (Weber ex Fr.) Fckl.—K; *Lamprospora* sp.—K; *L. discoidea* (Henn. & Nym.) Seaver—K; *Lasiobolus equinus* (Müll. ex Fr.) Karst.—K; *"Paxina" fusicarpa* (Ger.) Seaver—K; *"P." semitosta* (B. & C.) Seaver—K; *Peziza* spp.—K; *P. atrovinosa* Cke.—K; *P. gerardii* Cke.—K; *P. ?michelii* Boud.—K; *P. succosa* Berk. —K; *P. succosella* LeGal & Romagn.—K; *P. trachycarpa* Currey—K; *Psilopezia* sp.—K; *Pustularia cupularia* (L. ex Fr.) Fckl.—K; *Sarcoscypha floccosa* (Schw.) Sacc.—K; *S. occidentalis* (Schw.) Sacc.—K,

L; *Scutellinia* spp.—K; *S. erinaceous* (Schw.) O. Kuntze—K; *S. scutellata* (L. ex Fr.) Lambotti—K; *Trichophaca pseudogregaria* (Rick) Boud.—K.

BASIDIOMYCETES: Heterobasidiomycetes, Pucciniales: *Cerotelium tanake* S. Ito on *Amphicarpa bracteata*—P; *Coleosporium asperum* (Diet.) Syd. on *Solidago altissima*—Z, *S. canadensis*—P, *S. ulmifolia*—P; *C. denticulatum* Hedgc. & Long on *Solidago graminifolium*—Z; *C. vernoniae* Berk. & Curt. on *Vernonia altissima*—Z; *Cronartium ribicola* J. C. Fisher on *Ribes cynosbati*—P; *Frommea obtusa* (Strauss) Arth. on *Potentilla simplex*—P, Z; *Gymnoconia peckiana* (Howe) Trott. on *Rubus allegheniensis*—P; *Gymnosporangium juniperi-virginianae* Schw. on *Juniperus virginiana*—P, on *Pyrus malus* (Indiana U. Campus)—Z; *Hyalopsora polypodii* (Diet.) Magn. on *Cystopteris fragilis*—Z; *Kuehneola uredinis* (Lk.) Arth. on *Rubus allegheniensis*—P, Z; *Puccinia circaeae* Pers. on *Circaea quadrisulcata*—P; *P. helianthi* Schw. on *Helianthus lactiflorus*—Z, on *H. divaricatus*—P; *P. marylandica* Lindr. on *Sanicula canadensis*—P; *P. menthae* Pers. on *Cunila origanoides*—Z, on *Monarda fistulosa*—Z; *P. punctata* var. *trogdolytes* (Lindr.) Arth. on *Galium triflorum*—Z; *P. recondita* Rob. on *Agrostis perennans*—P, on *Hystrix patula*—P; *P. schedonnardi* Kell. & Sw. on *Muhlenbergia schreberi*—Z; *P. smilacis* Schw. on *Smilax tamnoides*—Z; *P. tenuis* Burr. on *Eupatorium rugosum*—P; *Pucciniastrum agrimoniae* (Diet.) Tranz. on *Agrimonia gryposepala*—Z; *Uromyces hedysari-paniculati* (Schw.) Farl. on *Desmodium rotundifolium*—Z; *U. hyperici* (Spreng.) Curt. on *Hypericum* sp.—L; *U. spermacoces* (Schw.) Curt. on *Diodia teres*—Z; *U. triquetrus* Cke. on *Hypericum proliferum*—P; *Uromyces* sp. on *Carex platyphylla*—P, Z.

Tremellales, Tremellaceae: *Aporpium caryae* (Schw.) Teix. & Rog.—L, R; *Exidia nucleata* (Schw.) Burt—M; *E. recisa* Fr.—M; *Sebacina caesio-cinera* (Hoehm. & Litsch.) Rog.—L; *S. epigaea* (Berk. & Br.) Rea—L, M, R; *S. helvelloides* (Schw.) Burt—L; *S. incrustans* (Fr.) Tul.—L, M. R; *Tremella mesenterica* Fr.—M; *T. concrescens* (Fr.) Burt—H, L, M; *T. obscura* (Olive) Christensen—L; *Tremellodendron candidum* (Fr.) Atk.—M; *T. cladonia* (Schw.) Burt—M; *T. schweinitzii* (Pk.) Atk.—C, F, G, H, L, R; Dacrymycetaceae: *Dacrymyces deliquescens* (Mérat) Duby)—L, M, var. *deliquescens*—M; var. *ellisii* (Coker) Kennedy—L; var. *minor* (Pk.) Kennedy—L, M; *Dacryomitra nuda* (Berk. & Br.) Mass.—L; Tulasnellaceae: *Gloeotulasnella calospora* (Boud.) Rogers—L, M, R; *Tulasnella bifrons* Bourd. & Galz.—L, R; *T. violea* (Quél.) Bourd. & Galz.—M; Auriculariaceae: *Helicogloea lagerheimii* Pat.—L, R; Septobasidiaceae: *Septobasidium lila-*

cinoalbum Couch—E; Phleogenaceae: *Phleogena faginea* (Fr.) Lk. —J, R.

HOMOBASIDIOMYCETES: Polyporales, Thelephoraceae: *Aleurodiscus microsporus*—R; *A. nivosus* (Berk. & Curt.) Hoehn. & Litsch.—L; *Asteroma muscicolum* (Berk. & Curt.) Mass.—L; *Botryohypochnus isabellinus* (Fr.) Erikss.—L; *Byssocorticium atrovirens* (Fr.) Bond. & Sing.—L, R; *Corticium galactinum* (Fr.) Burt—D; *Cristella farinacea* (Pers. ex. Fr.) Donk—L; *C. sulphurea* (Pers. ex Fr.) Donk—L; *Dacryobolus sudans* (Alb. & Schw. ex Fr.) Fr.—L; *Hyphodontia arguta* (Fr.) Erikss.—L; *Hypoderma setigera* (Fr.) Donk—L; *Hypochnella violacea* Karst.—H; *Grandinia farinacea* (Fr.) Bourd. & Galz.—L, R; *Oliveonia fibrillosa* (Burt) Donk—L; *Pellicularia pruinata* (Bres.) Rogers & Linder—L; *P. vaga* (Berk. & Curt.) Rogers —L; *Peniophora accedens* Bourd. & Galz.—R; *P. affinis* Burt—L; *P. aspera* (Pers.) Sacc.—O; *P. cinerea* (Fr.) Cke.—R; *P. cremea* Bres. —L; *P. filamentosa* (Berk. & Curt.) Burt—L, R; *P. hydnoides* Cke. & Mass. in Mass.—L; *P. longispora* (Pat.) Hoehn.—L; *P. pubera* (Fr.) Sacc.—L; *Porotheleum fimbriatum* (Pers. ex Fr.) Fr.—L; *Stereum cinerascens* (Schw.) Mass.—O; *S. frustulatum* (Pers. ex Fr.) Fckl.— O, L, R; *S. ostrea* (Bl. & Nees) Fr.—O; *S. complicatum* Fr. (*S. rameale*)—O; *S. sericeum* Schw.—O, R; *S. striatum* Fr.—L; *Schizophyllum commune* Fr.—C; *Hymenochaete rubiginosa* (Schrad. ex Fr.) Lév.—C, L; *Thelephora albido-brunnea* Schw.—F, L; *T. anthocephala* Bull. ex Fr.—R; *T. caryophyllea* Schaeff. ex Fr.—F; *Tomentella atroviolascens* Litsch.—L; *T. coriaria* (Pk.) Bourd. & Galz.—L; *T. echinospora* (Ell.) Bourd. & Galz.—L; *T. pallidofulva* (Pk.) Litsch.—L; *Trechispora subtrigonosperma* (Rog.) Rog. & Jackson—L, R; *Tubulicrinis angusta* (Rog. & Were.) Donk—L; *Vararia investiens* (Schw.) Karst.—L, R; *V. effuscata* (Cooke & Ellis) Rogers & Jackson—O; *Xenasma praeteritium* (Jackson) Donk—L; *X. tulasnelloideum* (Hoehn. & Litsch.) Donk.—L; Meruliaceae: *Merulius aureus* Fr.—C; *Clavaria vermicularis* Fr.—L; *Clavicorona pyxidata* (Fr.) Doty—H, L; *Clavulina cinerea* (Fr.) Schroet.—H; *C. cristata* (Fr.) Schroet. (grey form)—H; *Clavulinopsis corniculata* (Fr.) Corner—L; *Ramaria stricta* (Fr.) Quél. —L; *Clavaria* spp. (most collectors); Hydnaceae (sensu lato): *Dentinum repandum* (L. ex Fr.) S. F. Gray—C, F, H, L; *Hericium coralloides* (Scop. ex Pers.) Banker—G; *Hydnellum velutinum* (Fr.) Banker (southern form)—H; *H. zonatum* (Batsch. ex Fr.) Karst.—F. H; *Odontia ciliolata* (B. & C.) Miller—O; *O. laxa* Miller—L; *O. spathulata* (Fr.) Litsch.—L; *Oxydontia setosa* (Pers.) Miller—D; *O. fragilissima* (Berk. & Curt.) Miller—L; *Steccherinum adustum* (Schw.) Banker—

C, H, L; *S. ochraceum* (Pers.) S. F. Gray—C, O; *S. rawakense* (Pers.) Banker—R; *S. rhois* (Schw.) Banker—F, H; Cantharellaceae: *Cantharellus cibarius* Fr.—A, F, J, L; *C. cinnabarinus* (Schw.) Schw. —A, F, H, J, L; Polyporaceae (sensu lato): *Antrodia mollis* (Sommerf. ex Fr.) Karst.—C; *Bjerkandera adusta* (Willd. ex Fr.) Karst.—D; *Cerrenella farinacea* (Fr.) Murr.—L; *Coriolus biformis* (Fr.) Ames —C, F, R; *C. nigromarginatus* (Schw.) Murr.—O; *C. versicolor* (L. ex Fr.) Quél.—F, R. O; *Coltricia cinnamomeus* (Jacq. ex Fr.) Murr. —C; *C. perennis* (L. ex Fr.) Murr.—C; *Daedalea aesculi* (Schw.) Murr.—G, R; *D. confragosa* Bolt. ex Fr.—F; *D. juniperina* Murr.—C; *D. quercina* (L. ex Fr.) Fr.—C; *Favolus alveolatus* (DC.) Quél.—C, O; *Fomes cajanderi* Karst.—G; *F. everhartii* (Ell. & Gall.) von Schrenck —D, O, R; *F. robiniae* (Murr.) Sacc. & Trott.—O; *F. scutellatus* (Schw.) Cke.—C, D; *F. ?robustus* Karst.—C; *Ganoderma applanatum* (Pers. ex Wallr.) Pat.—L; *Glocoporus dichrous* (Fr.) Bres.—C, F, O; *Grifola cristata* Pers. ex S. F. Gray—C; *Hapalopilus gilvus* (Schw.) Murr.—C, L, O, R; *H. nidulans* (Fr.) Karst.—F; *Heteroporus biennis* (Bull. ex Fr.) Lazaro—O; *Irpiciporus lacteus* (Fr.) Murr. (*P. tulipiferae*)—C, F; *Irpex cinnamomeus* Fr.—C; *Lenzites betulina* (L.) Fr. —C, F; *Melanoporia nigra* (Berk.) Murr.—D, L; *Phlebiella candidissima* (Schw.) W. B. Cke.—O; *Polyporus arcularius* Batsch. ex Fr. —C; *P. picipes* Fr.—C; *P. semipileatus* Pk.—C; *Poria alutacea* Lowe —D; *P. europa* (Karst.) Cke.—C; *P. ferruginosa* (Schrad. ex Fr.) Karst.—C; *P. hypolateritia* (Berk.) Cke.—C; *P. latitans* Bourd. & Galz.—C; *P. overholtsii* Pilát—L; *P. punctata* (Fr.) Karst.—D; *P. radiculosa* (Pk.) Sacc.—C; *P. subacida* (Pk.) Sacc., atypical—C; *P. subrufa* Ellis & Dearness—C; *P. unita* (Pers.) Cke.—C; *P. versipora* (Pers.) Rom.—O; *Poronidulus conchifer* (Schw.) Murr.—C, F, R; *Pycnoporus cinnabarinus* (Jacq. ex Fr.) Karst.—C, O; *Spongipellis fissilis* (Berk. & Curt.) Murr. (Bloomington)—D; *Trametes sepium* Berk.—O; *T. serialis* Fr.—D; *Truncospora ohiensis* (Berk.) Pilát— C, D, F, R; *Tyromyces albellus* (Pk.) Bond. & Sing.—L; *T. semisupinus* (Berk. & Curt.) Murr.—L; *T. semipileatus* (Cke.) Murr.— L, O; *T. spraguei* (Berk. & Curt.) Murr.—C, D, L, O.

Agaricales: Hygrophoraceae: *Hygrophorus conicus* Fr.—A, L; *H. miniatus* Fr.—A, L; Tricholomataceae: *Collybia butyracea* (Bull. ex Fr.) Quel.—H; *C. platyphylla* Pers. ex Fr.—H; *C. radicata* Fr.—A, D, H, L; *Clitocybe adirondakensis* (Pk.) Sacc.—A; *Marasmius* sp.—G; *M. siccus* (Schw.) Fr.—A; *Mycena leaiana* Berk.—G; *Asterophora lycoperdoides* (Bull.) Ditmar ex S. F. Gray—G; *Panellus stypticus* (Bull. ex Fr.) Karst.—C; *Pleurotus* sp.—G; Amanitaceae: *Amanita bispori-*

gera Atk.—A; *A. brunnescens* Atk.—L; *A. cokeriana* Sing.—L; *A. rubescens* (Pers. ex Fr.) Gray—G, H, R; *A. spreta* (Pk.) Sacc.—A; *A. ?virosa* Lam. ex Secr.—L; *A. vaginata* (Bull. ex Fr.) Quél. (white and tawny forms)—J, H, R; Agaricaceae: *Agaricus* sp.—L; *A. micromegethus* Pk.—A; Coprinaceae: *Coprinus micaceus* (Bull. ex Fr.) Fr.—G, J; Strophariaceae: *Kuehneromyces vernalis* (Pk.) Sing. & Smith—A; Cortinariaceae: *Cortinarius lilacinus* Pk.—A; Rhodophyllaceae: *Clitopilus novaboracensis* Pk.—A; Boletaceae: *Boletus chrysenteron* Bull. ss. Coker & Beers—H, L; *B. fraternus* Pk.—A; *B. griseus* Frost in Peck—A, H, L; *B. rubellus* Krombh. ssp. *sonsobrinus* Sing.—A; *Boletinellus merulioides* (Schw.) Murr.—A, H, L; *Gyroporus castaneus* (Bull. ex Fr.) Quél.—A; *Leccinium albellum* (Pk.) Sing.—A; *Phylloporus rhodoxanthus* (Schw.) Bres. ssp. *americanus* Sing.—A, L; *Pulveroboletus auriflammeus* (Berk. & Curt.) Sing.—A, H, L; *P. auriporus* (Pk.) Sing.—A; *P. retipes* (Berk. & Curt.) Sing.—A, H, L; *Tylopilus alboater* (Sing.) Murr.—A, H; *T. felleus* (Bull. ex Fr.) Karst.—A, H, L; *T. indecisus*—H, L; *T. tabacinus* (Pk.) Sing.—A; *Xerocomus subtomentosus* (L. ex Fr.) Quél.—L; Strobilomycetaceae: *Boletellus chrysenterioides* (Snell) Snell—A; *Strobilomyces floccopus* (Fr.) Karst.—A; Russulaceae: *Lactarius allardii* Coker—A; *L. cinereus* Pk.—H; *L. corrugis* Pk.—H, L; *L. fuliginosus* (Fr. ex Fr.) Fr.—G; *L. hygrophoroides* Berk. & Curt.—A; *L. indigo* (Schw.) Fr.—H; *L. piperatus* (L. ex Fr.) S. F. Gray—J, H, L; *L. subdulcis* (Bull. ex Fr.) Gray—H; *L. "subplinthogalus"* Coker—A; *L. subvelutinus* Pk., *L. volemus* (Fr.) Fr.—A, F, H, L; *Russula* spp.—L; *R. mariae* Pk.—J; *R. nigricans* Fr.—H.

GASTEROMYCETES: Phallales: Phallaceae: *Dictyophora duplicata* (Bosc.) Fischer—I; *Mutinus caninus* (Pers.) Fr.—L; Lycoperdaceae: *Calvatia craniiformis* (Schw.) Fr.—J; *Lycoperdon perlatum* Pers.—L; *L. pyriforme* Pers.—G, J; *L. subincarnatum* Pk.—L; Geastraceae: *Geastrum* spp.—J; *G. fimbriatum* Fr.—N; *G. saccatum* Fr.—L; Sclerodermatales: Sclerodermataceae: *Scleroderma aurantium* Pers.—G; *S. lycoperdoides* Schw.—L; Nidulariales: Nidulariaceae: *Crucibulum levis* (DC.) Kambly & Lee—C, J, L; *Cyathus* sp.—C; *C. striatus* Pers.—J, L, O.

FUNGI IMPERFECTI: (Alphabetical order regardless of family): *Alternaria* sp.—B; *A. dauci* (Kuehn.) Neergaard—S; *Amblysporium botrytis* Fres.—B; *Aspergillus clavatus* Desm.—B; *Berkleasmium concinnum* (Berk.) Fr.—N; *Calcarisporium arbuscula* Preuss—L; *Candida boidini* Ramirez—W; *Cladosporium herbarum* (Pers.) Lk.—B; *Costantinella athrix* Nannf. & Erikss.—L; *Cylindrosporium* sp. on *Prunus*

virginiana—P; *Dactylium dendroides* (Bull.) Fr.—L; *Epicoccum* sp.—B; *Fusarium* sp.—B; *Geotrichum* sp.—W; *Gibellula pulchra* (Sacc.) Cavara—L; *Gliocladium deliquescens* Sopp.—B; *G. fimbriatum* Gilman & Abbott—B; *Haplaria salicina* (Sacc.) Hoehn.—L; *Kloeckera apiculata* (Roess. emend. Klöcker) Janke—W; *Isaria* sp.—S; *Leptothyrium pomi* (Mont. ex Fr.) Sacc.—L; *Ozonium auricomum* Lk.—F, J; *Paecilomyces elegans* (Corda) Mason & Hughes in Hughes—B; *Penicillium cyclopium* Westling—B; *P. frequentens* Westling—B; *P. janthinellum* Biourge—B; *P. lilacinum* Thom—B; *P. nigricans* (Bainier) Thom—B; *P. roseo-purpureum* Dierck, *P.* sp.—B; *Pestalotia* sp.—B; *Polythrincium trifolii* Kae.—S; *Rhinotrichum curtisii* Berk.—L; *Rhopaloconidium asiminae* (Ell. & Morg.) Petrak on *Asimina triloba*—N; *Rhynchosporina tridentis* Sprague & Rogerson on *Triodia flava*—N; *Sphaerosporium lignatile* Schw.—N; *Sporoschisma saccardoi* Mason & Hughes—L; *Stephanoma strigosum* (Wallr.) Sacc. on *Humaria hemispherica*—N; *Taeniola alta* (Ehrenb.) Bon.—L; *Trichoderma viride* (Pers.) Fr.—B; and *Xenosporella larvalis* (Morg.) Linder—L.—CHESTER W. EMMONS, National Institutes of Health, Bethesda; GEORGE B. CUMMINS, Purdue University, Lafayette; and WM. BRIDGE COOKE, Robert A. Taft Sanitary Engineering Center, Cincinnati.

THE EFFECT OF STREPTOMYCIN ON THE GROWTH OF THE PLASMODIUM OF PHYSARUM POLYCEPHALUM [1]

In an attempt to study the action of streptomycin on *Physarum polycephalum* streptomycin sulfate was added to the medium. After some time it became evident that the growth characteristics of the plasmodium had been affected by the prolonged contact with the antibiotic. This note is to describe the phenomenon and show it is readily repeatable.

The plasmodia were grown in pure, bacterium-free cultures. Their sterility was tested by seeding pieces of plasmodium in Difco nutrient broth and Difco nutrient agar and in Difco A-C broth. The tubes were kept in an incubator at 25° C during a period not less than 10 days.

[1] This study was carried out at Princeton University under the direction of Prof. J. T. Bonner, to whom sincere acknowledgment is given for his counsel and inspiration during the course of the investigation and for his help in the preparation of the manuscript. I am also indebted to Dr. G. W. Martin, Dr. M. F. Filosa and Miss I. M. Morrow. This work was done on a Rockefeller Foundation fellowship which is gratefully acknowledged. Accepted for publication in December, 1959.

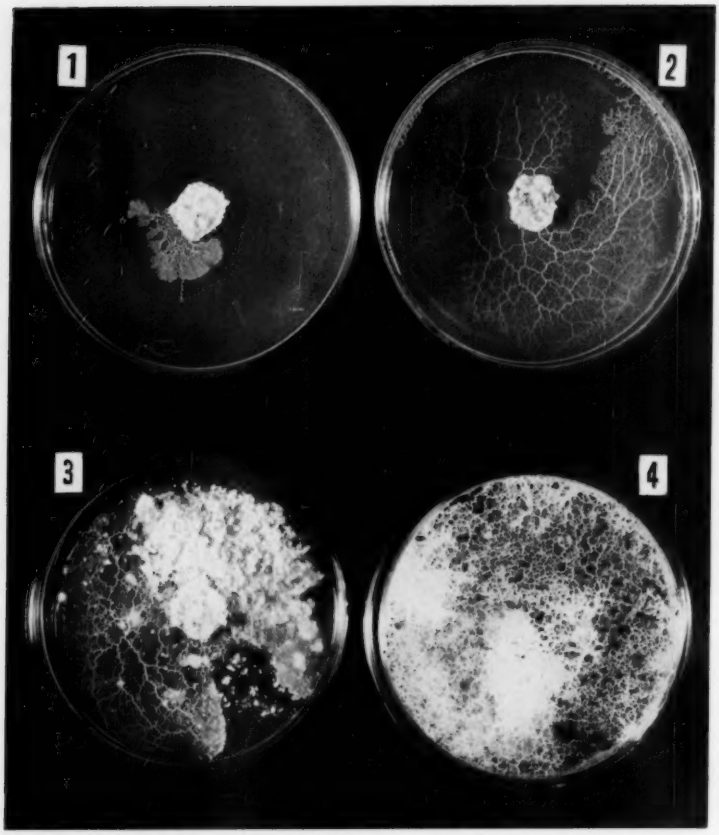

FIG. 1. Growth of an untreated plasmodium of *Physarum polycephalum* after 24 hours on Difco corn agar. FIG. 2. Growth of a streptomycin-modified plasmodium on Difco corn agar. FIG. 3. Growth of an untreated plasmodium after four days on oat agar. FIG. 4. Growth of a streptomycin-modified plasmodium which has completely covered the inside surface of the Petri dish. (In both experiments the initial inoculum of plasmodia was approximately of the same size. All plates were grown at 27°.)

Most of the experiments were done on a medium containing rolled oats in 1.5% agar. In some of the successive transfers Difco corn agar was used.

A piece of plasmodium was transferred to non-nutrient agar containing 5 mg per cc of streptomycin sulfate. It was incubated in the light at 22° for three weeks and then placed on a shelf in the laboratory.

Subcultures were made from this culture plate 2, 29, 31 and 35 days after the beginning of the experiment. The first three transfers gave normal growth in the streptomycin-free plates, but the 35-day transfer, which appeared whitish and unhealthy, gave a far more vigorous growth in the streptomycin-free culture plate (FIGS. 1–4).

This characteristic of rapid growth (which consists of both an increase of mass and faster spreading) was retained after seventeen transfers over a period of four months.[2]

The difference between the transformed plasmodia and the controls is especially evident at 27°, while at 21° the difference is negligible. The growth rate is neither increased nor decreased by a second period of treatment for one month on a fresh medium containing streptomycin.

The experiment was repeated using 10 and 20 mg per cc of streptomycin sulfate and in both cases similarly transformed plasmodia were induced if transfers were made when the plasmodia were degenerating and turning white. Negative results were obtained with penicillin, sigmamycin, mycostatin, erythromycin and amphotericin B.[3]

There are a number of aspects of these experiments that are in need of further investigation. It would be especially interesting to compare the normal and transformed strains for cytological and metabolic differences. It should be mentioned that in one streptomycin-modified strain there has been no fruiting even after repeated attempts involving 25 passages, provided the plasmodium remains free from microbial contamination. Therefore, the possibility that the modification affects the ability to fruit is another matter of interest and in need of more study.—WALDO R. LAZO, Escuela de Agronomía, Casilla 1004, Santiago, Chile.

ACRASIALES OF THE WEST INDIES

During the period of December 9, 1960, to January 6, 1961, the writer visited several islands in the Caribbean area in search of cellular slime molds. Of the 7 species that were found, several were quite common and one had not been previously described. Data on all spe-

[2] In the course of these experiments it was found that the untreated plasmodium itself exhibits antibiotic activity towards gram-positive and gram-negative bacteria. The streptomycin-modified strain has a weaker antibiotic activity.

[3] The author is indebted to Dr. Otto K. Behrens of E. Lilly and Co., who kindly furnished the erythromycin, and to Squibb and Sons Co. for the mycostatin and the amphotericin B.

cies except those in the genera *Protostelium* and *Acrasis* may be found in the reviews of E. W. Olive (**1902**) and Bonner (**1959**).

Guttulinaceae

Guttulinopsis vulgaris E. W. Olive. This species was described as being common on fresh cultures of various kinds of dung, yet there appears to be no report of it since its original description. The spores ("pseudospores") were described as irregularly spherical, and in one of the illustrations (Olive, **1902**, Fig. 16) a spore is shown that is concave on two sides. Spores with concave sides (1–3) are the most characteristic feature of all our collections of this species. This feature probably offers the best evidence that our collections are synonymous with Olive's species. The fruiting bodies are whitish, and each appears as a globose spore mass on a short stalk-like base. It is one of the most common of the cellular slime molds encountered by us both in the West Indies and in this country from the New England states to North Carolina. It seems to occur only on very fresh dung about two days after it is placed in a moist chamber. When isolated it grows well in agar culture with certain bacteria. Further studies of this species are planned in the near future. In the Caribbean area *G. vulgaris* occurred on dung of goat, mule and cow in Trinidad, Grenada, and Martinique.

Acrasis rosea L. S. Olive and Stoianovitch (**1960**). This attractive, pigmented cellular slime mold with catenulate spores has been reported previously only in the eastern part of the United States. Recently, Olive et al. (**1961**) have published the occurrence of natural variants within the species that differ from each other primarily in their food range. An isolate from Martinique (M-1) resembles our Race NC-18 from North Carolina in this respect, but on an albino mutant of *Rhodotorula mucilaginosa* the growth of amoebae remained nearly colorless instead of showing the characteristic orange color in mass. Also, the spores were a lighter pink than is typical. Later, after the isolate was returned to our laboratory and cultured for several months, pigmentation of the organism on albino *Rhodotorula* and other food microorganisms was found to be typical. In the West Indies *A. rosea* was found on dry pods of a leguminous herb and a leguminous tree in Grenada, and on dry pods of the green bean in Martinique.

Acytosteliaceae

Protostelium mycophaga L. S. Olive and Stoianovitch (**1960**). In morphology this is probably the simplest of the cellular slime molds.

The spherical spores are produced singly at the tips of slender acellular stalks. All cells contain an orange pigment. Recent studies indicate that it is one of the most common of the Acrasiales. In the Caribbean area it was isolated from leguminous pods, cotton bolls, dead flowers and decaying fruits of *Lantana,* dead flower umbels from a tree, and old flower stalks of a monocot; Grenada, St. Lucia and Martinique.

Protostelium sp. This undescribed species was first found by us on cow dung in North Carolina. It is a round-spored species that differs from *P. mycophaga* in lack of pigmentation and in having spore stalks that readily gelatinize in water. Also, it utilizes bacteria rather than fungi as food. The species was found on cow and goat dung in Grenada and St. Lucia. On a collection of fresh goat dung in St. Lucia it sporulated so abundantly that it gave a whitish bloom to the substrate.

In a forthcoming monograph of the genus *Protostelium* (Olive, 1962), the new fimicolous species will be described in addition to another new species with oblong spores from North Carolina, and the genus will be transferred to a family of its own.

Dictyosteliaceae

Dictyostelium mucoroides Brefeld. This well known species is very common in the West Indies, having been isolated from soil, humus, leguminous pods, ears and tassels of corn, and decaying breadfruit; Trinidad, Grenada, St. Lucia (including Pigeon Island) and Martinique.

D. purpureum E. W. Olive. This species closely resembles the foregoing, but may be distinguished from it by its purple spore masses. It was isolated 3 times from soil and humus in Grenada and St. Lucia (Pigeon Island).

Polysphondylium violaceum Brefeld. The compound fruiting bodies with whorls of secondary stalks and violaceous spore masses readily distinguish this attractive species. It was isolated 3 times in Grenada from soil, leguminous pods, and dead flowers of the cannon ball tree.

Sappinia, which appears to be common in the West Indies, is no longer considered a valid member of the cellular slime molds and is therefore not discussed here.

The writer is grateful to the American Philosophical Society for a research grant which made this study possible, and to Dr. William Beebe and Dr. Jocelyn Crane for their kind hospitality and for the use of the laboratory facilities of the New York Zoological Society at Simla, Trinidad.—LINDSAY S. OLIVE, Department of Botany, Columbia University, New York, N. Y.

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REVIEWS

PLANT PATHOLOGY: PROBLEMS AND PROGRESS, 1908–1958. Edited by C. S. Holton, G. W. Fischer, R. W. Fulton, Helen Hart and S. E. A. McCallan. 588 p., illus., 26 June 1959, University of Wisconsin Press, Madison. Price, $8.50.

The papers assembled in the ten parts of this volume were given at the Golden Jubilee Meeting of the American Phytopathology Society at the University of Indiana, Bloomington, August 24–28, 1958. The portions of probable interest to mycologists include Part II, a "Symposium on physiology of parasitism," Part III, a "Symposium on genetic approach to elucidation of mechanisms governing pathogenicity and disease resistance," Part VI, a "Symposium on soil microbiology and root disease fungi" and Part X, a "Symposium on epidemiology of plant diseases." Mycologists interested in genetics of fungi will find Part III useful enough to warrant obtaining the volume. Part VI will be of value to beginning students of soil fungi. In addition, the papers by Warcup, Lockhead, Stover and Wilhelm contain information not readily found elsewhere. Mycologists will find John Stevenson's paper in Chapter 2 of Part I on early history of applied mycology (phytopathology) informative.

The text has been carefully edited, with good illustrations including one colored plate. It is largely free from errors.

A few of the technical papers have been obviously reduced in length to keep the book in one volume size, while some papers in Part I are perhaps too long. In general, within the limits of a Jubilee volume, the editors have done a superlative job. The text is well worth owning.—RODERICK SPRAGUE.

DIE GATTUNG PHLEGMACIUM (SCHLEIMKÖPFE). ("DIE PILZE MITTELEUROPAS" BAND IV), by Meinhard Moser, p. 1–440, pls. A–F, and 32 color plates under separate cover. Edited by Deutsche Gesellschaft für Pilzkunde, Deutsche Botanische Gesellschaft, Verband Schweizerischer Vereine für Pilzkunde. Verlag Julius Klinkhardt, Bad Heilbrunn Obb. Price, DM 172.00.

Like the previous volumes, this work is based on original research by a competent specialist of the respective group of fungi. It has been a very good idea to approach the genus *Cortinarius* (or as some including M. Moser prefer, the group of very closely related genera entering the Friesian *Cortinarius*) step by step, viz. by editing an illustrated monograph on a regional scope, in this case central Europe. Nobody could have been better equipped to bring such an undertaking to a successful end than Moser, foremost expert in *Cortinarius* questions in Europe, and perhaps in the world, and author of a much used and appreciated field manual of European Agaricales and Gastromycetes, as well as one of the pioneers of mycorrhiza research, both basic and applied. This latter fact is important in this case because it is now reasonably well established that virtually all species of *Phlegmacium* are formers of ectotrophic mycorrhiza with forest trees and salicaceous and fagaceous shrubs of the alpine and tundra zones, associating themselves with the roots of the most important forest trees, especially Fagales and conifers of Europe whereby a complex organism now often called ectotroph results. The different specialization and the different contribution towards the nutrition and stimulation of growth of the ectotroph as represented by each species of fungi, and each race of that species, make the Phlegmacia extraordinarily important as a biological tool in the hands of the modern forester not only in central Europe but in many parts of the world. It is fitting that in Moser's book ample data are given which refer to these relations particularly the chapters on pure culture of the Phlegmacia, mutations, influence of physiological and ecological factors on the development of these fungi and some data on the biochemistry involved. The Phlegmacia are much less important for the mycophagist although some species are edible, and no poisonous species are known in central Europe. It adds credit to the publisher that he has undertaken the task of publishing monographs of this kind continuing a series which until now contained mainly mushrooms of general public appeal as far as the European amateur is concerned. This is also the first volume written by a non-German author.

In the taxonomic part, 166 species and numerous varieties and forms are completely described (including full anatomical and chemical data) and keyed out in clear keys which include, for greater usefulness outside central Europe, also such "exotic" species as may be expected to be found in Europe. There is also a French version of the key, and Latin diagnoses of the species described as new.

The genus is divided into several sections, subsections and "Kreise" (= stirpes). The synonymy is given in strongly abbreviated but appar-

ently complete form. The scientific names are etymologically explained. The habitat is particularly well described. The rules of nomenclature are generally taken into consideration. The colored plates are mostly the work of the author. They are both in original execution and reproduction among the best that have been published. About 200 species, varieties and forms are thus illustrated in color, the most complete atlas ever published in this field. This is particularly useful in this group of colorful, large showy agarics where a good illustration can help so much in identifying a form at hand. Four plates (195 figures) with spore drawings will add to the usefulness of the work, and the plate (F) with spore print colors opens the way for a new approach, until now generally neglected.

It would be presumptuous to attempt to criticise some of the minor taxonomical or nomenclatorial solutions offered even though there might be some occasional details which other specialists of the group would have approached differently. The important thing is that the work has been done carefully, thoroughly, with the type method in mind, and with full documentation. This will make it very valuable and stimulating far beyond the geographical limits set by the author and the title. The author as well as the publisher should be congratulated by everybody interested in the progress of mycology, and this volume outstanding for its good print, attractive appearance, and interesting contents, should be present in every mycological library.—R. SINGER.

DIE ROSTPILZE MITTELEUROPAS, by Ernst Gäumann. Beiträge Kryptogamenflora Schweiz, Vol. 12, 1407 p., 1075 figs. 1959. Price, $14.50.

In this voluminous book Dr. Gäumann describes 6 families and 826 species of the rust fungi, lists the host plants, discusses the biology of each, appends 79 pages of literature citation, and provides host and fungus indexes. The book presents the most recent and the most complete record of the Uredinales of central Europe.

In common with some other European mycologists, Gäumann apparently looks upon any named species as a "sacred cow" to be handed on to posterity without scrutiny or violation. He states that the species concept used is a narrow one, and it is. Arthur's "Manual of the Rusts in United States and Canada" covers a larger area and a more varied flora with 84 fewer species (and 969 fewer pages). But this is not a

fair comparison because I may not know what Gäumann means by a species. He states (transl.) that ". . . to facilitate organization, related species and little species (Kleinarten) are assembled in species groups. A 'Formenkreis' in the foregoing sense is more than just a broad Linnean species; it includes rather all those systematic units which supposedly belong together genetically, that is in the ideal cases." This is much the same as the concept of correlated species, used by Arthur, in that microcyclic species are placed with the supposed macrocyclic parent. Often the primary emphasis is given to the aecial host. This breaks the leaf-rust complex into 7 "Formenkreisen" and 31 species (or are they Kleinarten?). Included as No. 13 is "Formenkreis der *Puccinia rubigo-vera* (D. C.) Wint." but the SPECIES *rubigo-vera* is not described—only 5 components. The "Formenkreis" organization (there are 34 in *Uromyces*, 104 in *Puccinia*, for example) generally puts related forms together, but it can be overdone. *Puccinia purpurea* Cke. turns up as kin of *Puccinia sorghi* Schw., although the 2 have nothing in common except aecial hosts in the genus *Oxalis; Uromyces leptodermus* Syd. (as *U. setariae italicae*), with equatorial pores, falls into the group of *Uromyces dactylidis* Otth, all of whose components have many scattered pores. But anomalies of this sort are inherent in any system and are not especially objectionable.

While I disagree strongly with a species concept that fragments broad but recognizable species, as Gäumann's does, the records are here. One can put the pieces together, and the data on life cycles are abundant and valuable.

The book suffers most from a sort of ancestor worship. This is reflected in the retention of just about every binomial proposed in Europe in the last 75 years. *Calyptospora* is retained as a genus, but with this statement: "*Calyptospora* ist nur eine opsis-Form der Gattung *Thekopsora;* man würde sie also, wäre sie nicht schon beschreiben, heute nicht mehr aufstellen." And the descriptions are based when possible on those of Fischer (1904) and Klebahn (1914) because "Es wäre müssig, ihre Diagnosen verbessern zu wollen." One result of this is an inconsistent and insufficient use of the germ pores of the urediospores, features of real utility in speciation. Sometimes the number and distribution are given; sometimes only the number. If there are many, one may assume a scattered arrangement; if few, one assumes an equatorial or a zoned arrangement, but there are exceptions. Certainly a beginner is in no position to make these assumptions. Further, Gäumann has disinterred Klebahn's system of specific names, so there are many trinomials. Whether the trinomial is hyphenated depends upon the origin of the

epithet. Thus, the specific epithet of (*Melampsora*) *euphorbiae dulcis* Otth, an autoecious species, is not hyphenated because it derives from *Euphorbia dulcis* L.; but the epithet of (*Melampsora*) *ribesii-purpureae* Kleb., a heteroecious species, is hyphenated because it combines a part of the name of the aecial host (*Ribes* spp.) and a part of the name of a telial host (*Salix purpurea* L.). Gäumann also transgresses the Rules by using the oldest name, whether given to the perfect state or to an imperfect state, but apparently he accepts 1801 as the starting date.

The usual listing of synonyms is omitted. This is probably a generally progressive move but in this instance students who use the manual must accept the names as gospel and many of them certainly are not. The legitimate alternatives ought to be given, if for no other reason than to show the pitfalls into which nomenclature may fall if it follows the International Code of Botanical Nomenclature (and most other botanists). Dr. Gäumann believes that it is unfortunate that the nomenclature specialists have brought disorder to the authority designations of the rust fungi. He has taken positive steps to compound the disorder.—GEORGE B. CUMMINS.

COLORED ILLUSTRATIONS OF FUNGI OF JAPAN, by R. Imazeki and T. Hongo. viii, 181 p., 68 colored and I–VIII black and white plates. 1957. Osaka, Japan. Price, 1200 yen (approx. $3.35).

This volume is an attempt to acquaint the Japanese mycophagist with the mushrooms of Japan, those species which may be eaten without toxic effect, and methods of collecting and preparing them.

For the person who does not read Japanese, the plates speak for themselves. Sixty plates with 342 figures illustrate with reproductions from water colors 342 species of fleshy and woody fungi in both the Ascomycetes and Basidiomycetes. An additional 8 plates reproduced from colored photographs illustrate the remainder of the 406 species described. Eight plates in black and white illustrate techniques of collection and preservation, special conditions of growth and the techniques of mushroom culture.

While the text is arranged in such a way that plates will be near the description, the book is based on a definite systematic treatment derived from at least two sources. Some representative Ascomycetes are illustrated and described but primary emphasis is on Basidiomycetes. Those species treated are included in the Agaricales and the Aphyllophorales.

The system used for the Agaricales is based on that of Singer's recent work. The families of the Aphyllophorales include those recently proposed by Imazeki.

Because it is thought that less than half the fleshy fungi of Japan are known to science, directions for collection, preservation and description are quite detailed.

A word of warning should be given to the mycophagist who does not read Japanese. If the illustrations are relied on for identification of mushrooms, the identification should be double checked in areas not specifically covered by the geographic range of the book. In spite of this problem, which applies to any text on mushroom identification, the book is beautifully done and would be an interesting addition to any mycologist's library. (Assisted in translation by George Matsuura.)—WM. BRIDGE COOKE.

MANUSCRIPT

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